# A BIOMETRICAL STUDY OF CROSSING OVER. ON THE MECHANISM OF CROSSING OVER IN THE THIRD CHROMOSOME OF *DROSOPHILA MELANOGASTER*[1]

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## INTRODUCTION

This paper is an attempt to analyze the current theories of crossing over by a study of its normal fluctuating variations in a particular chromosome. It is the direct outgrowth from, and indeed in some respects, a supplement to the studies of crossing over made during the past few years in this laboratory. During this investigation, however, the problem has been approached from a different point of view and has made use of rather more adequate methods.

The problem and the point of view taken may be best defined by considering a few of the already known facts concerning crossing over in Drosophila. If one counts separately the offspring of a large number of back-crossed females heterozygous for a large number of factors, there

[1] A contribution from the Zoölogical Laboratory of COLUMBIA UNIVERSITY.

may be formed from the resulting data characteristic curves of variation of the number of breaks for each region of the chromosomes which were contained in the females under consideration. The precise form of these curves, as well as the location in them of the value calculated from the offspring of any particular female, is the result of two basic variables, environment and heredity;—environment, in that the conditions surrounding the germ cells of one female may be more favorable to crossing over in one region or mayhap in the whole chromosome than the conditions in another female; heredity, in that a gene may, when substituted for another gene in the same locus, influenced in a marked way the crossing over in a given region.

Little has been done toward a direct analysis of factors such as these in their bearing on the contending views of the mechanism behind the crossing-over phenomena. Yet clearly such an analysis offers one of the best means of extending our knowledge and furnishes critical evidence. The study of interindividual variation offers a way by which the problem of the mechanics of crossing over may be attacked.

Specifically, the direction of the attack on the general problem is that of the analysis of the variation curve in terms of its component individuals. A given individual in the frequency distribution may show a particularly high rate of crossing over for one section of the chromosome. Will it show the same high rate for other sections and, if so, will it also show this proportionately high rate for the double crossing over including these two regions? Does the substitution of other genes for those normally present affect the crossing over in an individual concerned in the formation of our variation curve? These examples will give a definite idea as to the general manner of approach to the problem of variation in crossing over followed in this paper.

## MATERIAL AND METHODS

Most of the data contained in this paper were collected during the years 1915-'16 and 1916-'17 that the author has been a member of the Zoölogical Laboratory at COLUMBIA, the rest was obtained at Cold Spring Harbor, Long Island, during the summer of 1916.

To make the conditions as nearly constant as possible with regard to temperature, all flies were bred and reared in an incubator controlled by a thermostat to maintain a temperature of 25° C. Even with this precaution, it is realized that this is not altogether satisfactory, for in summer the outside temperature often rises higher than 25° C. However this rise is slight, and it is thought that the conditions have been

maintained so constant that temperature variations may be said to be negligible.

It is probable that food has no effect on crossing over. But as a change was made from fermented banana to an artificial food mixture of starch, sugar, peptone, yeast, and water, this factor will be discussed in connection with the data.

The factors used throughout were those which lie in the third chromosome. Enumerated in the order of their position, they are sepia ($s_e$), Dichaete ($D'$), curled ($c_u$), peach ($p^p$), spineless ($s_s$), hairless ($H'$), sooty ($e^s$), and rough ($r_o$). These factors are arranged in the chromosome as seen in diagram 1.

Several distinct sets of experiments were made, using different combinations of these factors. In all cases they were made as back-crosses of a heterozygous female to a male homozygous for the recessives carried by the female. The specific kind of cross that was used in each experiment will be given in connection with a discussion of the data.

For the data on the effect of selection on crossing over, all of the matings were made strictly brother and sister. For the rest of the data this practice has not always been followed, although it generally has been. In all cases the record for the output from each female, represents the offspring of that female mated to a single male. The time allotted for the hatching of the eggs which were laid is in every case ten days after the first fly hatches. Thus the count of a given female is obtained by counting all flies which hatch during the ten days following the emerging of the first offspring from the pupa case.

It hardly seems necessary to say that contamination was carefully watched as a source of error in the data. In every case all of the triple crossovers were tested to be sure that there were no mistakes. Any other cultures which gave extreme results were bred from to test the result. From this it is thought that the cultures included in these data are free from contamination and non-virginity errors.

In recording data, each region of the chromosome may be designated in one of two ways. Thus $\overbrace{\underset{D'}{s_e \quad\quad s_s \quad e^s \; r_o}}^{1 \quad 2 \quad 3 \quad 4}$ the first region would be either $s_e$ $D'$ or 1; the second region $D'$ $s_s$ or 2, etc. In this way the regions are designated from left to right numerically as 1, 2, 3, 4, etc. The double crossover may also be recorded as $s_e$ $D'$ and $D'$ $s_s$ where a break occurs in the two regions sepia Dichaete and Dichaete spineless simultaneously, or it may be recorded as 1, 2 (break in region 1 and break

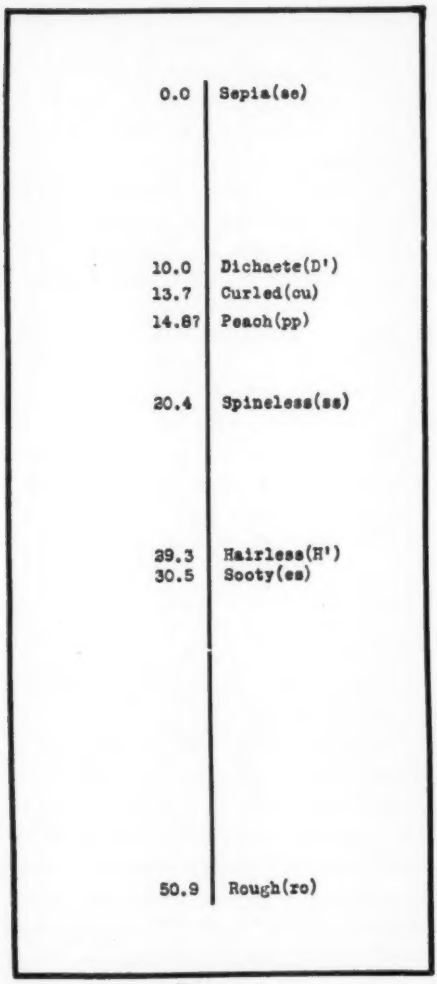


DIAGRAM 1

in region 2). The triple crossovers may likewise be recorded as 1, 2, 3, for one including the first three regions of the chromosome. Thus by an extension of this method all possible crossovers are recorded.

The biometrical methods used in the analysis of the statistical material are in general not different from those commonly in use. A few remarks may not be out of place, however, in regard to the computation

of some of the constants in this paper. In the calculation of the standard deviation SHEPPARD's correction for the second moment was not used as it is evident to anyone studying the distribution that there is no approach to high contact at one end of the distribution at least. In the back-cross test of heterozygous females, carrying the genes for sepia, curled, spineless, sooty, rough, in one chromosome, and for dichaete, and hairless in the other, by males homozygous for sepia, curled, spineless, sooty, and rough, all of the calculations were made from ungrouped frequency distributions. In the formation of the correlation tables all the data have been punched on cards and sorted, first into the frequency distribution for the first region, then the classes sorted into the frequencies for the second region to form the correlation surfaces. This sorting has all been done by the machine made by the Tabulating Machine Company. All of the calculation was done on one of the common calculating machines. It is hoped that there are no errors remaining in the computations, although it is impossible to be absolutely sure in a work as large as this that slight arithmetical slips have not gone by unnoticed.

## THEORETICAL ASPECTS OF THE PROBLEM OF THE MECHANISM OF CROSSING OVER

Before proceeding to the direct analysis of the problem of crossing over, it may be well to consider what the different theories of crossing over should give as observed results in a theoretically perfect experiment. The theories to account for crossing over which are now extant may be reduced to two.[2] The first of these, brought forward by BATESON and PUNNETT, as the reduplication theory, attempts to account for crossing over as a differential rate of division in germ-cell formation. The second takes as its fundamental postulate a twisting of the chromosome threads in loose twists.

If we carry the analysis of what would be expected on the reduplication theory to include, besides the single separation, those double separations of coupled factors, we would expect only such correlation between single separations and the successive double separations as would be brought about by their being correlated to the same thing. In other words, we would expect this relationship to vanish when we used partial correlations to measure directly the single separations and double

[2] In view of the recent criticism by STURTEVANT and BRIDGES of the hypothesis to account for crossing over brought forward by GOLDSCHMIDT, it seems to me wise to await the reply before considering it further.

separations. This is just what we would not expect on the twisting hypothesis of crossing over. Let us consider the case of a fixed point of twist having a known variation around a mean ratio (a) from the fixed point for the second twist. Now, if we take successive ratios of crossing over along this chromosome, with a break at one fixed point, what is the likelihood of another simultaneous break in successive regions as we progress along the chromosome away from the fixed point? Surely, it will increase to the mode of our frequency curve of the ratio for twist, and diminish from that toward the further end. Thus one of the strongest pieces of evidence that can be given for the twisting hypothesis to account for crossing over will be given if we can show that there is such a rise and fall in single and double correlations.

There are, unfortunately, some difficulties in our data which should be pointed out. In the first place, it is impossible to limit our first break to a fixed point because there are not enough good factors close enough together to do this. It is necessary, therefore, to take a small segment of the chromosome from which to measure. The successive regions taken to divide the frequency distribution of twisting have to be uneven intervals and further the interval in one of our segments ($e^s\ r_o$) has to be quite long. These are physical difficulties which I see no way of overcoming. They are not difficulties which in any way vitiate the conclusions, however, for in every case the effect is such that it subtracts from the numerical value of the coefficients measuring the relationship. Thus conclusions drawn from these coefficients have a big margin of safety.

These difficulties should, however, be kept constantly in mind in weighing the value of the evidence. Specifically, in our data, region $e^s\ r_o$ is poorly suited to this study because it is so long that should twists occur between 25 and 30 units apart the second twist might fall in either of two regions ($s_eD'$ or $D'_s$), depending on whether the first one is near $e_s$ or $r_o$. The mid-regions are also not well suited to this study as the regions on either side are not long enough to enable the mode of the curve to appear, if the modal frequency of twisting is about 25 units. The $s_e\ D'$ region considered in connection with the rest of the data is, however, well suited to the study, for here the first region is short and so located at the end of the chromosome that it has the whole length of the chromosome for the other twist to fall. Consequently, it is to that region which we will pay most attention in our subsequent analysis. Some other difficulties, such as genetic variations of crossing over, which if present materially influence our conclusions, will be the first to receive consideration.

## FREQUENCY DISTRIBUTIONS OF THE VARIATION IN PERCENT OF SINGLE AND DOUBLE CROSSING OVER IN THE THIRD CHROMOSOME OF DROSOPHILA

The frequency of the percentage of crossing over for the various regions is shown in tables 1 and 2, both in absolute figures and in percentages.

TABLE 1

*Percentage of single crossing over* $(\frac{s_e \quad s_s \quad c^s \quad r_o}{D'})$.

| Percent crossing over | Region 1 | | Region 2 | | Region 3 | | Region 4 | |
|---|---|---|---|---|---|---|---|---|
| | Frequency | Percent | Frequency | Percent | Frequency | Percent | Frequency | Percent |
| 0-2 | 2 | .83 | 2 | .83 | 1 | .41 | | |
| 2-4 | 4 | 1.66 | 10 | 4.16 | 9 | 3.75 | | |
| 4-6 | 18 | 7.50 | 30 | 12.51 | 18 | 7.50 | | |
| 6-8 | 29 | 12.08 | 51 | 21.26 | 45 | 18.76 | 3 | 1.25 |
| 8-10 | 44 | 18.34 | 57 | 23.76 | 57 | 23.76 | 2 | .83 |
| 10-12 | 55 | 22.93 | 31 | 12.92 | 44 | 18.34 | 5 | 2.09 |
| 12-14 | 38 | 15.84 | 27 | 11.25 | 29 | 12.08 | 13 | 5.41 |
| 14-16 | 30 | 12.51 | 16 | 6.66 | 19 | 7.91 | 15 | 6.25 |
| 16-18 | 13 | 5.41 | 9 | 3.74 | 11 | 4.59 | 38 | 15.84 |
| 18-20 | 3 | 1.25 | 5 | 2.09 | 4 | 1.66 | 34 | 14.17 |
| 20-22 | 3 | 1.25 | 1 | .41 | 3 | 1.25 | 48 | 20.00 |
| 22-24 | 1 | .41 | 1 | .41 | | | 28 | 11.67 |
| 24-26 | | | | | | | 23 | 9.58 |
| 26-28 | | | | | | | 17 | 7.08 |
| 28-30 | | | | | | | 7 | 2.92 |
| 30-32 | | | | | | | 5 | 2.09 |
| 32-34 | | | | | | | | |
| 34-36 | | | | | | | 1 | .41 |
| 36-38 | | | | | | | 1 | .41 |
| Total | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 |

In engaging in any discussion of the distributions and the interrelations between them, it seemed advantageous to have the physical constants, mean, standard deviation, and coefficient of variation, before us. In the calculations of these constants SHEPPARD'S correction for the second moment was not used.

A number of interesting points are brought out by this table:

1. It will be seen that the crossing-over ratio for $D's_s$ obtained by summation of the values for $D'c_u$ and $c_us_s$ that the sum is only 8.388 percent, as against 9.483 percent for the cross which does not contain

TABLE 2

*Percentage of double crossing over* $\left(\frac{s_0 \quad s_0 \quad e^a \quad r_0}{D'}\right)$.

| Percent double crossing over | Region 1, 2 | | Region 1, 3 | | Region 1, 4 | | Region 2, 3 | | Region 2, 4 | | Region 3, 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Frequency | Percent | Frequency | Percent | Frequency | Percent | Frequency | Percent | Frequency | Percent | Frequency | Percent |
| 0.0-0.5 | 96 | 40.00 | 92 | 38.31 | 35 | 14.59 | 152 | 63.34 | 67 | 27.91 | 154 | 64.17 |
| 0.5-1.0 | 45 | 18.76 | 26 | 10.84 | 14 | 5.84 | 42 | 17.50 | 31 | 12.92 | 39 | 16.25 |
| 1.0-1.5 | 27 | 11.25 | 38 | 15.84 | 40 | 16.66 | 26 | 10.83 | 41 | 17.09 | 31 | 12.93 |
| 1.5-2.0 | 30 | 12.51 | 30 | 12.51 | 38 | 15.84 | 11 | 4.59 | 32 | 13.33 | 5 | 2.09 |
| 2.0-2.5 | 16 | 6.66 | 19 | 7.92 | 33 | 13.75 | 5 | 2.09 | 24 | 10.01 | 6 | 2.50 |
| 2.5-3.0 | 16 | 6.66 | 18 | 7.51 | 20 | 8.33 | 1 | .41 | 13 | 5.41 | 2 | .83 |
| 3.0-3.5 | 3 | 1.25 | 5 | 2.09 | 20 | 8.33 | 1 | .41 | 13 | 5.41 | 1 | .41 |
| 3.5-4.0 | 3 | 1.25 | 5 | 2.09 | 14 | 5.84 | 2 | .83 | 11 | 4.59 | 1 | .41 |
| 4.0-4.5 | | | 1 | .41 | 12 | 5.00 | | | 2 | .83 | | |
| 4.5-5.0 | 4 | 1.66 | 3 | 1.25 | 5 | 2.09 | | | 5 | 2.09 | | |
| 5.0-5.5 | | | 2 | .83 | 4 | 1.67 | | | | | | |
| 5.5-6.0 | | | | | 1 | .41 | | | | | 1 | .41 |
| 6.0-6.5 | | | | | 2 | .83 | | | | | | |
| 6.5-7.0 | | | 1 | .41 | | | | | 1 | .41 | | |
| 7.0-7.5 | | | | | | | | | | | | |
| 7.5-8.0 | | | | | | | | | | | | |
| 8.0-8.5 | | | | | | | | | | | | |
| 8.5-9.0 | | | | | 2 | .83 | | | | | | |
| Total | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 | 240 | 100.00 |

TABLE 3

*Physical constants for the frequency distributions of the third chromosome.*

| Region | Mean | Standard deviation | Coefficient of variation |
|---|---|---|---|
| $s_e$ $D'$§ | 10.900 ± .166 | 3.807 ± .117 | 34.923 ± 1.199 |
| $s_e$ $s_s$* | 20.383 ± .276 | 6.332 ± .201 | 31.003 ± 1.074 |
| $s_e$ $e^s$* | 30.458 ± .361 | 8.282 ± .263 | 27.189 ± .917 |
| $s_e$ $r_o$* | 50.858 ± .420 | 9.654 ± .306 | 18.623 ± .624 |
| $D'$ $c_u$† | 2.845 ± .297 | 2.680 ± .210 | 58.956 ± 5.885 |
| $D'$ $s_s$§ | 9.483 ± .170 | 3.907 ± .120 | 41.201 ± 1.468 |
| $D'$ $e^s$* | 19.558 ± .258 | 5.916 ± .188 | 30.335 ± 1.034 |
| $D'$ $r_o$* | 39.958 ± .330 | 7.568 ± .240 | 18.513 ± .624 |
| $c_u$ $s_s$† | 5.543 ± .186 | 1.677 ± .131 | 40.823 ± 1.636 |
| $s_s$ $H'$† | 9.862 ± .251 | 2.263 ± .177 | 23.558 ± .875 |
| $s_s$ $e^s$§ | 10.075 ± .165 | 3.785 ± .117 | 37.570 ± 1.310 |
| $s_s$ $r_o$* | 30.475 ± .266 | 6.103 ± .192 | 19.427 ± .651 |
| $H'$ $e^s$† | 1.173 ± .258 | 2.323 ± .182 | 62.980 ± 6.414 |
| $e^s$ $r_o$§ | 20.400 ± .216 | 4.957 ± .153 | 24.300 ± .791 |
| $s_eD'$ and $D's_s$§ | 1.065 ± .045 | 1.029 ± .032 | 96.651 ± 5.040 |
| $s_eD'$ and $s_ee^s$§ | 1.273 ± .041 | .950 ± .029 | 74.636 ± 3.341 |
| $s_eD'$ and $e^sr_o$§ | 2.170 ± .063 | 1.458 ± .045 | 67.178 ± 2.853 |
| $D's_s$ and $s_ee^s$§ | .609 ± .027 | .613 ± .019 | 100.766 ± 5.401 |
| $D's_s$ and $e^sr_o$§ | 1.498 ± .053 | 1.210 ± .037 | 80.792 ± 3.777 |
| $s_ee^s$ and $e^sr_o$§ | .613 ± .029 | .672 ± .021 | 109.750 ± 6.240 |

* Compound constants calculated from the separate components by summation.
† Calculated from ungrouped frequencies of table A (Appendix, p. 241).
§ Calculated from grouped frequencies of table D (Appendix, pp. 243-247).

the gene for $c_u$. Likewise, in the summation of the values $s_sH'$ and $H'e_s$ there is quite a considerable difference from the result obtained in the cross without $H'$ (11.035 to 10.075). It remains for a further section of this paper to discuss whether or not these differences are significant.

2. It will be noted that there is a very high coefficient of variability in practically every ratio, this variability being greatest when the mean crossing over ratio is small. Thus, it may be said that when dealing with factors which separate only rarely there is to be expected great fluctuation in the value of the ratio of crossovers to the total number of flies.

3. It is further to be noted that the variability in the number of double breaks is markedly higher than when the variability of the single break is considered. This high variability is no doubt due in part to the small absolute number of double crossovers which are expected. However, this does not in any measure account for the whole of it. From the table

we can safely say that double crossing over is an extremely variable character.

4. A comparative view of this variability will give us a better basis for judging of its real magnitude. A constant is so named because it has a low variability, and as this variability becomes greater , its action is measured by so-called laws. Now, it will be interesting to compare some of the morphological characters which more nearly approach physical characters and some more nearly physiological characters with the values for crossing over.

TABLE 4

*Variation constants for various characters.*

| Subject | Character | Coefficient of variation | Authority |
|---|---|---|---|
| Poland-China swine | Size of litter | 27.41 | SURFACE (1909) |
| Man | Number of children | 48.14 | POWYS (1905) |
| English males | Heart weight | 22.22 | GREENWOOD and BROWN (1913) |
| Cattle | Rev. maximum daily milk yield | 18.00 | GAVIN (1913) |
| Domestic fowl | Shell weight of eggs | 13.86 | CURTIS (1914) |
| English | Length of skull | 3.31 | MACDONELL (1904) |
| Domestic fowl | Breadth of egg | 3.29 | PEARL (1914) |
| Drosophila | Single crossing over | 18.51-58.96 | This paper |
| Drosophila | Double crossing over | 67.18-109.76 | This paper |

A glance at this table suffices to show how much crossing over in different females varies. Even the lowest values stand well up among the characters which are more purely physiological in character and the highest value is much above that which is ordinarily found even in the physiological characters. Such a high variability demands explanation, and it will be the function of a succeeding section of the paper, where the data has been collected for it, to attempt such explanation.

## VARIATION IN CROSSING OVER BETWEEN TWO FIXED POINTS IN EXPERIMENTS CONTAINING OTHER INTERMEDIATE GENES

In undertaking a discussion of crossing over, a matter of prime importance is the question whether or not a change in the genes between two fixed points influences the amount of crossing over between these two fixed points.

It has already been shown by STURTEVANT (1917) and MULLER (1916) that there are disturbing factors in the second and third chromosomes which reduce the crossing over of the factors located in their re-

spective chromosomes. In every case these disturbing factors have quite a considerable effect, as, for example, the cutting down of crossing over from about 50 percent to 1 percent. Now the question arises, do all factors influence crossing over? Is an effect on crossing over as much a function of a gene as the eye color or the body color that is given to an animal by its presence? A partial answer to this problem may be had by a comparison of the crossing-over value for two sets of data in which it is known that in the first set a given gene is present which is not present in the second set. To this end the following data were collected in which heterozygous females of the composition indicated for each distribution were crossed with males homozygous for the recessives carried by the female.

It will be seen on examination of these tables that they differ from each other in having a different set of factors run in combination with certain common factors. Thus, table 5 differs from table 6 in being formed from a cross which has a chromosome carrying dichaete sub-

TABLE 5

*Genes,* $\frac{s_o \quad s_s \quad e^s \quad r_o}{D'}$.

| Non-crossovers | Single crossovers | | | | Double crossovers | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 |
| 17,171 | 2,208 | 2,211 | 2,639 | 5,163 | 292 | 392 | 629 | 132 | 413 | 125 |

| Triple crossovers | | | | Quadruple crossovers | Total |
|---|---|---|---|---|---|
| 1, 2, 3 | 1, 2, 4 | 1, 3, 4 | 2, 3, 4 | 1, 2, 3, 4 | |
| 11 | 39 | 17 | 13 | 1 | 31,456 |

TABLE 6

*Genes,* $\frac{s_o \quad s_s \quad e^s \quad r_o}{H'}$.

| Non-crossovers | Single crossovers | | | | Double crossovers | | | | | | Triple crossovers | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 1,2 | 1,3 | 1,4 | 2,3 | 2,4 | 3,4 | 1,2,3 | 1,2,4 | |
| 1459 | 378 | 175 | 29 | 436 | 31 | 8 | 89 | 10 | 10 | 2 | 3 | 1 | 2631 |

TABLE 7

*Genes,* $\frac{D'\ p^{p}\ s_{8}\ e^{8}\ r_{o}}{}$.

| Non-crossovers | Single crossovers | | | | Double crossovers | | | | | | Triple crossovers | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 1,2 | 1,3 | 1,4 | 2,3 | 2,4 | 3,4 | 1,2,3 | 1,2,4 | 1,3,4 | |
| 1220 | 92 | 263 | 312 | 366 | 15 | 25 | 30 | 9 | 32 | 11 | 1 | 4 | 1 | 2381 |

TABLE 8

*Genes,* $\frac{s_{e}\quad c_{a}}{D'}\quad\frac{s_{8}\quad e^{8}\quad r_{o}}{H'}$

| Non-crossovers | Single crossovers | | | | | | Double crossovers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 1,2 | 1,3 | 1,4 | 1,5 | 1,6 | 2,3 | 2,4 | 2,5 | 2,6 |
| 4664 | 534 | 112 | 319 | 689 | 77 | 1320 | 22 | 25 | 55 | 14 | 150 | 16 | 22 | 2 | 46 |

| Double crossovers | | | | Triple crossovers | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3,4 | 3,5 | 3,6 | 4,6 | 1,2,3 | 1,2,5 | 1,2,6 | 1,3,4 | 1,3,6 | 1,4,6 | 2,3,6 | 3,4,6 | 3,5,6 | |
| 3 | 1 | 44 | 37 | 1 | 1 | 1 | 1 | 3 | 1 | 5 | 1 | 1 | 8167 |

stituted for one carrying hairless. Table 5 differs from table 8 in having a chromosome carrying dichaete substituted for one carrying both dichaete and hairless.

This, then, gives the data necessary to test out the previous question, Is an effect on crossing over as much a part of the function of a gene as the character produced by it? For if the gene is not an integral part of the mechanism, but is simply carried along by it, it would be expected that a gene's presence would have no effect on crossing over. But if allelomorphs are granules of varying physical characteristics in the chromosome and crossing over takes place by the breaking of finely spun-out twisted threads, it would be expected that one type of granule substituted for another type of granule would affect the position and number of breaks in a chromosome, much the same as the breaking of the strands of a wire cable in a given place is influenced by whether German silver or steel occupies that place. All that is necessary is to compare the constant elements of these distributions as they would have been, supposing

that the gene in question had not been there. To compare the cross dichaete heterozygous with hairless heterozygous all that is necessary is to reduce the distribution of table 5 to what it would have been in a cross $\frac{s_e \ s_s \ k \ e^s \ r_o}{}$ neglecting dichaete and hairless as in table 9. As it was impossible to follow the kidney factor ($k$) in the presence of rough, it was not counted.

TABLE 9

| Formula of back-crossed female | Reduced formula | 0 | 1 | 2 | 3 | 1, 2 | 1, 3 | 2, 3 | 1,2,3 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| A. $\frac{s_e \ s_s \ e^s \ r_o}{D'}$ | $\frac{s_e \ s_s \ e^s \ r_o}{}$ | 17463 | 4419 | 2650 | 5202 | 524 | 1042 | 126 | 30 | 31456 |
| B. $\frac{s_e \ s_s \ e^s \ r_o}{H'}$ | $\frac{s_e \ s_s \ e^s \ r_o}{}$ | 1469 | 381 | 197 | 443 | 39 | 89 | 12 | 1 | 2631 |

TABLE 10

*Reduction of crossing over of table 9 to single crossover in each region.*

| Formula of back-crossed female | Reduced formula | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| A. $\frac{s_e \ s_s \ e^s \ r_o}{D'}$ | $\frac{s_e \ s_s \ e^s \ r_o}{}$ | 17463 | 6015 | 3330 | 4600 |
| B. $\frac{s_e \ s_s \ e^s \ r_o}{H'}$ | $\frac{s_e \ s_s \ e^s \ r_o}{}$ | 1469 | 420 | 249 | 543 |

Without going into further detail, the eight possible arrangements of these five tables were made for the comparison of the effect of their different factors on the crossover ratios between the common factors. The measure used for this comparison was the well-known $\chi^2$ test of PEARSON. Since none of these were theoretically fitted frequency curves, the comparison was made only between the non-crossovers and single crossovers (obtained by summation) of each distribution. In this way the danger of $q$ being small in the case of double crossovers is avoided. In the following table are given the results of the comparisons of these distributions.

From this it is seen that even taking the greatest probability (that of the two curves $D'$ heterozygous and $H'$ heterozygous being the same), the odds against any of these curves coming from the same population are all more than three times the probable error (25 to 1). The disturbance

TABLE 11

| Distributions compared | $\chi^2$ | P |
|---|---|---|
| $\frac{Se\ Su\ k\ c^a\ r_o}{D'}$ and $\frac{Se\ Su\ k\ e^s\ r_o}{H'}$ | 8.37 | Less than .039859 |
| $\frac{Se\ Su\ k\ c^a\ r_o}{D'}$ and $\frac{D'\ b^p\ Su\ k\ e^s\ r_o}{}$ | 234.76 | $1/10^{60}$ |
| $\frac{Se\ Su\ k\ c^a\ r_u}{D'}$ and $\frac{Se\ Cu\ Su\ k\ e^s\ r_o}{D'\qquad H'}$ | 82.81 | $1/10^{34}$ |
| $\frac{Se\ Cu\ Su\ k\ c^a\ r_o}{D'\qquad H'}$ and $\frac{Se\ Su\ k\ e^s\ r_o}{H'}$ | 29.94 | .000005 |
| $\frac{Se\ Cu\ Su\ k\ c^a\ r_o}{D'\qquad H'}$ and $\frac{D'\ b^p\ Su\ k\ e^s\ r_o}{}$ | 307.05 | $1/10^{64}$ |

does not necessarily confine itself to the region occupied by the gene, as a study of the distributions will show. It may be anywhere and its effect may be more or less pronounced. In general, however, the effect is not as great as that of the previously found modifiers for crossing over. From this it follows that each gene has been accompanied by a disturbance in the crossing-over mechanism. This disturbance, it seems to me, may be due to the difference in the strains and stresses set up in the chromosome by different types of particles.

The essential conclusions to be drawn from this section of the work is that only those experiments containing as nearly as possible the same genes can be used in any critical study of crossing over and that the amount of crossing over between two fixed genes is a variable quantity, depending on the genes which are present. This does not mean that the crossing-over ratio is not a good means of measuring the position of the factors in a chromosome, it merely means that the scale from experiment to experiment may vary. Thus we should carefully consider the factors present in every experiment.

### DOES FOOD OR SEASON INFLUENCE CROSSING OVER?

To test this, it becomes necessary to divide our records (table D) at the places where a change of food occurred. The distributions resulting may then be compared by means of the previously described test, $\chi^2$, for similarity. Table 12 gives the distributions and the $\chi^2$ with the resulting probabilities that the various distributions can come from random sampling. Thus the $\chi^2$ value of 9.78 obtained by the comparison of the distribution resulting from the use of fermented banana and of a starch-

sugar mixture for food indicates that once in 22 such trials as good or worse a fit would be expected.

TABLE 12

| | | 0 | 1 | 2 | 3 | 4 | Total | Distributions compared | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|
| Fermented banana | I | 4456 | 866 | 744 | 779 | 1691 | 8536 | I & II | 9.78 | .045 |
| Starch-sugar mixture | II | 4944 | 1009 | 922 | 959 | 1824 | 9658 | II & III | 5.78 | .219 |
| | III | 5797 | 1193 | 962 | 1132 | 2159 | 11244 | I & III | 6.91 | .147 |

This gives a fair chance that all three curves are samples from the same population as would be expected *a priori*. The second and third distributions are the much better-fitting divisions. From this it may be concluded that the food used has little or no effect on crossing-over. Since the divisions may also represent divisions for different seasons, it follows from this that seasons have little or no effect on crossing over unless one takes the doubtful stand that the effect of food and season exactly counterbalance each other.

## CROSSING OVER IN RELATION TO MODIFYING FACTORS

It is too obvious to require experimental demonstration that modifying factors for crossing over would influence in a marked way the data obtained for crossing over in a large number of females where such modifying factors were present.

In an earlier section of this paper it has been shown that such a crossing-over disturbance does occur when known and accompanying unknown genes are introduced between fixed points. Now, it is conceivable that there are unknown genes in any cross which may cause crossing-over disturbances and will be distributed unevenly in the female whose offspring are counted. This uneven distribution will make the results from such a cross heterogeneous. The test of whether or not the material to be used in the succeeding study of crossing over is homogeneous will be dealt with in this section.

If we select only the females giving the lowest total percent of crossing over in the chromosome in question, we should lower the percent of crossing over if modifying factors are present. Such a selection experiment has been performed, the selection continuing for six generations of strictly brother-and-sister matings. In some respects these data are unsatisfactory. The chief difficulty lies in the few individuals that it was

possible to include in a given generation. In this way a large individual variation is possible which may in some cases obscure the results. However, taken as a whole, I think it will be found to answer the question, for the constants are rather uniformly the same in showing no effect of selection.

The material chosen for this selection experiment was the cross, male homozygous for sepia, spineless, kidney, sooty, rough, mated to a female heterozygous dichaete and sepia, spineless, kidney, sooty, rough. This cross is chosen as it is to be the cross used in the study of the mechanism of crossing over which is to follow. The reductions of the data to their means and correlations for each given generation are tabulated in table 13.

TABLE 13

| Generation | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Mean total of crossing over | 37.97±.970 | 47.81±1.274 | 49.67±1.929 | 49.03±1.272 | 55.75±1.09 |

| | 1 and 2 | 2 and 3 | 3 and 4 | 4 and 5 |
|---|---|---|---|---|
| Parent-and-offspring correlations | .1033±.1423 | .1595±.1756 | .3360±.1348 | —.2107±.1177 |

A like experiment has been made for selection of females with high percent of crossing over; the results are seen in table 14.

TABLE 14

| Generation | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Mean | 58.07±1.150 | 51.52±1.717 | 52.57±.587 | 49.85±2.769 | 51.16±.929 | 54.80±2. |

| | 1 and 2 | 2 and 3 | 3 and 4 | 4 and 5 | 5 and 6 |
|---|---|---|---|---|---|
| Parent-and-offspring correlations | —.0641±.1105 | .0297±.3369 | .1500±.2484 | —.1348±.1912 | —.0780±.2021 |

It is evident from this data that while the probable errors are large, the data are in accord in showing no effect of selection. This is even more striking when one considers the individual pedigrees where one generation may jump to the other extreme from its parent.

In this connection, it seems to me especially instructive to compare the correlations obtained by MACDOWELL (1917) during his selection experiment with those we have here. After selection had been continued for

more than fifty generations, the parent and offspring correlations in one set of experiments for the bristle number were —0.1436 for the males and —0.1378 for the females, or correlation of about the same magnitude as those in the experiment given above. Thus we see that in actual magnitude the correlations are about the same size for our experiment as those

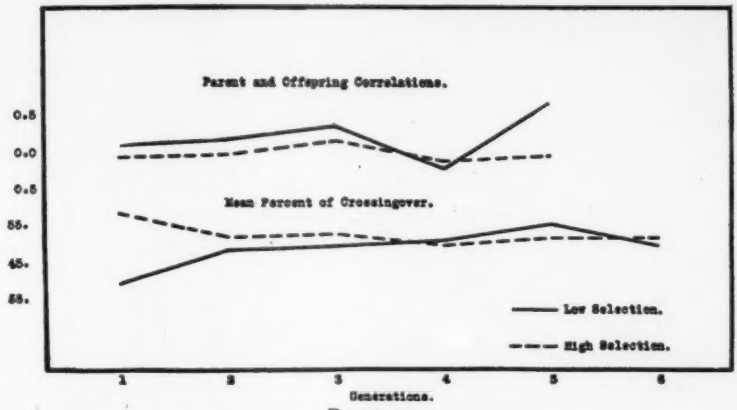


DIAGRAM 2

for an experiment carried on for some fifty-four generations where it was shown that selection had no appreciable effect after the first six generations. It seems a justifiable conclusion to be drawn from the above, the origin of the stock from a single pair, and the subsequent long inbreeding, that the records are homogeneous and without heterozygous modifying factors for either reduction or increase in crossing over. The crossing-over mechanism is, then, working in a system of events controlled only by the mechanism used in crossing over for the particular set of factors.

## ON THE RELATION BETWEEN NUMBER OF OFFSPRING AND VIABILITY OF THE FACTOR COMBINATIONS USED

As an explanation of the difference of expected from obtained ratios in some crosses, the hypothesis of differential viability has been proposed. This is on the face of it a legitimate hypothesis for such results, as it is known that some combinations of factors are less viable than others. Now, in a bottle of a large number of flies, where crowding takes place, it is an easy step to consider that in the competition more of the less viable combinations of factors die. Since this cause might be a disturbing

influence in the ratios obtained in these experiments, if one combination of factors were less viable than another, it becomes necessary to test for disturbances of linkage due to inviability. If it is considered that the number of flies produced by a bottle is a suitable measure of its condition, then if there is a disturbance of the linkage due to the viability in any given combination with variations in food or crowding, it would be expected that the ratios obtained for such bottles would be correlated. If we find no such correlation it is justified to conclude that no such difference in viability of factors remained unbalanced in our experiments. Table 15 shows the correlation for the given combinations of factors and the bottle output.

TABLE 15

| Region | Correlation | Region | Correlation |
|---|---|---|---|
| 1 | .0670 ± .0433 | 1, 3 | .1115 ± .0429 |
| 2 | —.0622 ± .0433 | 1, 4 | .0299 ± .0434 |
| 3 | .1174 ± .0429 | 2, 3 | —.0410 ± .0434 |
| 4 | .0275 ± .0434 | 2, 4 | —.0658 ± .0433 |
| 1, 2 | .0119 ± .0434 | 3, 4 | —.0436 ± .0434 |

Thus it will be seen that only in two cases does the correlation run as high as 0.1. Even this is only slightly above two times the probable error and therefore cannot be considered as significant. We may conclude with safety, then, that in no combination of factors which resulted from the crosses that were used did unbalanced differential viability exist.

The general conclusions which may be drawn from the first part of this paper are:

1. Crossing over between two fixed points is highly variable both relatively and absolutely.

2. A change in genes between two or more fixed points in the third chromosome may be accompanied by a slight disturbance of the crossing-over ratios between these fixed points.

3. The food used had no effect on crossing over.

4. It is highly probable that there were no unequally distributed modifying factors for crossing over at work in our data for the back-crossed females heterozygous for dichaete and sepia, spineless, kidney, sooty, rough, to be used for the critical study of crossing over in the succeeding section.

5. There is no effect of unbalanced differential viability resulting from any of our combinations of factors.

### ON THE RELATION OF CROSSING OVER TO POSITION

In any study of the crossing-over mechanism an adequate analysis of the problem must include a knowledge of the relation between the total crossing over in one part of the chromosome and the total crossing over in the remaining regions. Toward the solution of this phase of the problem the data in table D (page 243) have been collected. The cross of a homozygous sepia spineless kidney sooty rough male mated to a heterozygous female sepia spineless kidney sooty rough on one side, dichaete on the other, is the same as that used in our selection experiment where the stock was shown to be free from factors modifying the crossing-over ratios. For want of space the formed correlation tables have been omitted. All correlation coefficients have been calculated by the usual formula,

$$r = \frac{s(xy)}{N\sigma_x\sigma_y}$$

The problem presents some difficulties as the above formula does not give the true correlation as uninfluenced by other associated variables. For obtaining these it is necessary to resort to the use of partial correlations. The fundamental correlations are shown in table 16 with their probable errors calculated by the help of the tables edited by PEARSON (1914).

TABLE 16

*Coefficients of correlation for total crossing over in the different regions of the chromosome, together with those for number of offspring.*

| Section | Coefficient of correlation | Section | Coefficient of correlation |
|---|---|---|---|
| 1 and 2 | 0.7136 ± .0213 | 2 and 4 | 0.5985 ± .0280 |
| 1 and 3 | 0.7888 ± .0164 | 2 and T* | 0.6628 ± .0244 |
| 1 and 4 | 0.7426 ± .0195 | 3 and 4 | 0.7410 ± .0196 |
| 1 and T* | 0.8108 ± .0149 | 3 and T | 0.8335 ± .0133 |
| 2 and 3 | 0.6761 ± .0236 | 4 and T | 0.8782 ± .0100 |

* T stands for the total offspring output per female.

In every case the correlations are high and many times their probable errors. This table furnished us the material for calculation of the correlations between the different regions when the effect of difference in number of offspring per mating, on the correlation between two given chromosome regions, is eliminated through the use of partial correlations.

TABLE 17
*Partial correlations deduced from table 16.*

| Regions correlated | Partial correlations (Total constant) |
|---|---|
| 1 and 2 .T | .4019 ± .0365 |
| 1 and 3 .T | .3492 ± .0382 |
| 1 and 4 .T | .1093 ± .0430 |
| 2 and 3 .T | .2988 ± .0396 |
| 2 and 4 .T | .0458 ± .0435 |
| 3 and 4 .T | .0341 ± .0435 |

The correlations range from 0.4019 to 0.0341, from nine times the probable error to insignificance. An interesting relationship is apparent in this range of correlation. Considering any given region correlated with the remaining regions, the algebraic value of the correlation coefficients is greatest for the first left-hand region and diminishes toward the right hand. This is clearly brought out in table 18, together with the differences and their probable errors calculated by the usual difference formulae.

TABLE 18
*Correlation and differences for the single crossovers of successive chromosome segments.*

| Regions | Coefficient of correlation | Difference | Regions | Coefficient of correlation | Difference |
|---|---|---|---|---|---|
| 1 and 2 .T | .4019 | | 3 and 1 .T | .3492 | |
| 1 and 3 .T | .3492 | .0527 ± .0528 | 3 and 2 .T | .2988 | .0504 ± .0550 |
| 1 and 4 .T | .1093 | .2399 ± .0575 | 3 and 4 .T | .0341 | .2647 ± .0584 |
| 2 and 1 .T | .4019 | | 4 and 1 .T | .1093 | |
| 2 and 3 .T | .2988 | .1031 ± .0539 | 4 and 2 .T | .0458 | .0635 ± .0611 |
| 2 and 4 .T | .0458 | .2530 ± .5876 | 4 and 3 .T | .0341 | .0117 ± .0615 |

The significance of these differences becomes apparent by a comparison with their probable errors. In each case the relation of the crossing over between the 1 and 3, 2 and 3, and 3 and 2, with the fourth 1 and 4, 2 and 4, and 3 and 4, is quite significant. The other values do not have such great significance, some of the greater difference in the case of the fourth region may be due to its greater length. The consistency of the relationship leads one to suspect that it is more than chance, even though the differences are not great in comparison with the probable errors.

The fact that all of the eight differences between adjoining sections arranged in order from left to right are positive when the likelihood *a priori* is equal, as to whether any given difference shall be an excess or defect, is greatly in favor of the view that this is not a chance relationship, but is one brought about by some inherent cause in the mechanism of crossing over. Taken at its face value, this graded scale of correlation means that the conditions in a given female favorable to a given grade of crossing over in the first region is in a less degree favorable to crossing over in the second region and to a still less degree to crossing over in the regions to the right of the second.

It is further brought out that the distribution of the single crossovers for the different segments of the chromosome is by no means a random sample. They are associated variates. This conclusion is important in a number of ways. The chief among these is that in treating any expectation for double crossovers as the product of the single crossovers, either as just the single observed crossovers or as the single crossovers obtained by summation, we are committing a grave error. The error lies in the fact that we do not take into consideration that they are in general correlated variates.

Because of the importance of this correlation and to further test its generality another experiment was performed, using a larger number of factors. The cross used for this was a male homozygous for sepia, curled, spineless, kidney, sooty, rough, mated to a female heterozygous for dichaete, hairless on one side, and sepia, spineless, kidney, sooty, rough, on the other. It is realized that this distribution is not comparable with the preceding one, yet should the mechanism of crossing over be the same, the manner of crossing over in the two cases should be alike, even though the absolute values were different.

The correlations for this cross are given below. It is realized only too keenly that they are based on rather small numbers; as measured by the standard of the probable error, however, they are significant. Not only that, but taken in consideration with the preceding data it is believed as greater numbers are gathered that the correlations will remain practically the same except for a little tendency to smooth. Table 19 gives the correlation for the successive regions calculated from the ungrouped frequencies by the use of the ordinary formula.

By examination of this table it is seen that it substantiates a former conclusion that the crossing-over values for various sections of the chromosome may be, and generally are, correlated variates. This corre-

TABLE 19

*Coefficients of correlation for the successive regions of the third chromosome.*

| Section | Coefficient of correlation | Section | Coefficient of correlation |
|---|---|---|---|
| $s_o$ $D'$ and $D'$ $c_u$* | 0.5792 ± .0677 | $D'$ $c_u$ and $e_s$ $r_o$ | 0.0640 ± .1004 |
| $s_o$ $D'$ and $c_u$ $s_s$ | 0.3100 ± .0911 | $c_u$ $s_s$ and $s_s$ $H'$ | 0.1945 ± .0969 |
| $s_o$ $D'$ and $s_s$ $H'$ | 0.4440 ± .0809 | $c_u$ $s_s$ and $H'$ $e^s$ | 0.2752 ± .0932 |
| $s_o$ $D'$ and $H'$ $e^s$ | —0.0070 ± .1003 | $c_u$ $s_s$ and $e_s$ $r_o$ | 0.2023 ± .0967 |
| $s_o$ $D'$ and $e_s$ $r_o$ | 0.1920 ± .0971 | $s_s$ $H'$ and $H'$ $e^s$ | —0.2003 ± .0967 |
| $D'$ $c_u$ and $c_u$ $s_s$ | 0.2224 ± .0958 | $s_s$ $H'$ and $e^s$ $r_o$ | 0.1194 ± .0994 |
| $D'$ $c_u$ and $s_s$ $H'$ | 0.3970 ± .0849 | $H'$ $e^s$ and $e^s$ $r_o$ | —0.2364 ± .0952 |
| $D'$ $c_u$ and $H'$ $e^s$ | —0.1330 ± .0990 | | |

*The total number of offspring for each section held constant by the method of partial correlations.

lation runs as high as 0.5792 and drops to —0.2364. The correlation for the $H'e^s$ distance is seen in most cases to be abnormal. This is probably due to the fact that the ratio for crossing over of this section is rather small in absolute magnitude and since there are only a few crossovers expected with the number of individuals small, the crossing-over values are subject to considerable variation. For this reason where more individuals are included in the data this discrepancy will straighten out and fall in line with the observations previously made. This conclusion is justified, it is thought, for when the data are plotted the curve of the successive regional correlations of the smaller series onto the curve of the successive regional correlations for the larger series of data, the curve of the larger series is seen to bisect the fluctuations of the smaller series as would be expected if they were samples of like populations governed by the same mechanical laws. Thus, barring the slight modifications of the double-crossover values caused by the kind of intermediate factors present, in general the relationship previously established is seen to hold.

Thus, there is a correlation between the crossovers in a given region and the crossovers in successive regions. In general, this relation between crossing over in the various regions is greatest between the region toward the left-hand end of the chromosome and the region under consideration. That is, high crossing over in region BC is more likely to be correlated with high crossing over in AB than in the region of CD.

### ON THE RELATION BETWEEN CROSSING OVER IN ONE REGION AND CROSSING OVER INCLUDING TWO REGIONS AT A TIME

The question having most immediate bearing on the mechanism of crossing over is: What is the relation between crossing over in one region and crossing over including two regions at a time, for the successive segments of the chromosome? The importance of the question lies in the fact that if it can be shown that the value of the double crossovers falls in the ordinary cocked-hat frequency curve, it indicates that there is a definite modal length for maximum amounts of double crossing over. Such a modal length is accounted for by the twisting hypothesis of crossing-over as due to the chromosome threads lying across each other in loose twists during the stage at which crossing over takes place. If it can be shown that the double crossing-over ratios do rise and fall for each of the successive segments of the chromosome studied, it not only strengthens the twisting hypothesis, but puts the only other existing hypothesis of reduplication in the forced position of adopting another co-hypothesis to account for the fact, as has been shown in the previous discussion of the theory. To make a study of the crossing-over relationship secure and to be sure that our deductions are not based on a false groundwork, it is necessary to carefully consider how the experiment is performed. All factors that are to be compared should be put together in the same experiment and only data known to be alike in modifying factors used. Such data are at hand in the data on back-crossed females contained in our records for the back-crossed female sepia, spineless, kidney, sooty, rough, on one side, the dominant dichaete on the other, as has been shown by the previous parts of this paper. Before beginning the mathematical analysis of the data it may be well to consider some general aspects of the material as presented. There is one difficulty which must be taken into consideration when considering all measurements containing the region $e^s r_o$, that is, this region is about twice the value of the other regions under consideration. Unfortunately, it was impossible to get this region broken up into smaller parts, for as yet no good factor is known to occupy this region. This high ratio of crossing over means that if the length of the double crossing over is of a value such that the two extremes of the long $e^s r_o$ region fall so as to include two segments, the data will have a bimodal distribution and our observed correlation will have a distorted value. This possible distortion should be kept in mind when considering the data, and in general the main conclusions should rest on observations of the shorter distances.

The first difficulty confronted is the fact that the single crossovers in the various regions of the chromosome are correlated variates partly dominated in their position of breaking by some such cause as the plasma surrounding them. Thus it becomes necessary in a critical experiment to determine the mode of distribution of the breaks in a chromosome, to keep the single-crossover correlations constant throughout the length of the chromosome in females used in the experiment (constant with regard to its quality to influence double crossing over). Since the influence of this relation has been measured by the correlations of the different regions of the chromosome with one another, a mathematical universe may be formed to measure the double crossovers as they would have occurred in an experiment where the surroundings of the chromosomes are constant. In other words, in this way the obstacle of differential single crossing over as regards the various regions of the chromosome is removed. When this is done it is possible to measure the relation that exists in the double crossovers of the successive regions of the chromosome, knowing that it is only the effect of the chromosome mechanism that is studied.

To the end of establishing such a universe, it is necessary to obtain the relationship between the successive regions of the chromosome for double crossing over as they occur in a universe affected by the correlation of the single crossovers for the different regions. To this end the data given in table 20 were collected.

TABLE 20

*Correlations between single and double crossovers and number of offspring per mating.*

| Doubles | Region 1 correlation | Region 2 correlation | Region 3 correlation | Region 4 correlation | Total offspring |
|---|---|---|---|---|---|
| 1, 2 | 0.5578 ± .0300 | 0.6336 ± .0260 | 0.4421 ± .0350 | 0.3634 ± .0378 | 0.3857 ± .0371 |
| 1, 3 | 0.7724 ± .0176 | 0.6239 ± .0266 | 0.7073 ± .0218 | 0.5640 ± .0297 | 0.6180 ± .0269 |
| 1, 4 | 0.6483 ± .0253 | 0.4804 ± .0335 | 0.5730 ± .0292 | 0.6202 ± .0268 | 0.6224 ± .0267 |
| 2, 3 | 0.1795 ± .0421 | 0.3572 ± .0380 | 0.3285 ± .0388 | 0.1375 ± .0427 | 0.1734 ± .0422 |
| 2, 4 | 0.4810 ± .0335 | 0.6258 ± .0265 | 0.4472 ± .0348 | 0.4907 ± .0330 | 0.4249 ± .0357 |
| 3, 4 | 0.4220 ± .0358 | 0.4269 ± .0356 | 0.5336 ± .0311 | 0.4489 ± .0348 | 0.3830 ± .0372 |

Before tabulating the constants which will be necessary to the final study, the establishing of the linearity of regression for the tables from which the fundamental correlations of table 16 and table 20 have been deduced, is necessary. The constants to determine this are given in table 21.

TABLE 21
*Criteria for linearity of regression.*

| Characters correlated | $r$ | $\eta$ | $\eta - r$ | $\Sigma_m$ | $\eta^2 - r^2$ |
|---|---|---|---|---|---|
| T and 1 | .8108 ± .0149 | .8275 ± .0137 | .0167 | .3287σ | .0274 ± .0143 |
| T and 2 | .6628 ± .0244 | .6733 ± .0238 | .0109 | .2353σ | .0140 ± .0102 |
| T and 3 | .8335 ± .0133 | .8439 ± .0125 | .0104 | .2611σ | .0173 ± .0114 |
| T and 4 | .8782 ± .0100 | .8847 ± .0094 | .0066 | .2127σ | .0115 ± .0093 |
| T and 1, 2 | .3857 ± .0371 | .4085 ± .0363 | .0228 | .2674σ | .0181 ± .0115 |
| T and 1, 3 | .6180 ± .0269 | .6563 ± .0248 | .0383 | .4019σ | .0488 ± .0187 |
| T and 1, 4 | .6224 ± .0267 | .6394 ± .0258 | .0172 | .2993σ | .0227 ± .0123 |
| T and 2, 3 | .1734 ± .0422 | .1971 ± .0418 | .0237 | .1862σ | .0088 ± .0081 |
| T and 2, 4 | .4249 ± .0357 | .4436 ± .0350 | .0187 | .2532σ | .0162 ± .0109 |
| T and 3, 4 | .3830 ± .0372 | .4008 ± .0365 | .0178 | .2347σ | .0140 ± .0102 |
| 1 and 2 | .7136 ± .0213 | .7307 ± .0203 | .0172 | .2722σ | .0247 ± .0135 |
| 1 and 3 | .7888 ± .0164 | .7993 ± .0157 | .0105 | .2241σ | .0167 ± .0112 |
| 1 and 4 | .7426 ± .0195 | .7472 ± .0192 | .0046 | .1437σ | .0069 ± .0072 |
| 1 and 1, 2 | .5578 ± .0300 | .5752 ± .0291 | .0174 | .2438σ | .0197 ± .0121 |
| 1 and 1, 3 | .7724 ± .0176 | .7788 ± .0171 | .0064 | .1730σ | .0099 ± .0086 |
| 1 and 1, 4 | .6483 ± .0253 | .6728 ± .0239 | .0245 | .3123σ | .0324 ± .0154 |
| 1 and 2, 3 | .1795 ± .0421 | .2227 ± .0414 | .0431 | .2288σ | .0174 ± .0113 |
| 1 and 2, 4 | .4810 ± .0331 | .4920 ± .0330 | .0110 | .1797σ | .0107 ± .0089 |
| 1 and 3, 4 | .4220 ± .0351 | .4796 ± .0335 | .0576 | .3957σ | .0519 ± .0190 |
| 2 and 3 | .6761 ± .0230 | .6847 ± .0231 | .0086 | .1662σ | .0117 ± .0094 |
| 2 and 4 | .5985 ± .0280 | .6238 ± .0266 | .0253 | .2702σ | .0309 ± .0150 |
| 2 and 1, 2 | .6336 ± .0260 | .6647 ± .0243 | .0211 | .3086σ | .0404 ± .0171 |
| 2 and 1, 3 | .6293 ± .0266 | .6580 ± .0247 | .0341 | .3213σ | .0437 ± .0177 |
| 2 and 1, 4 | .4804 ± .0335 | .5289 ± .0314 | .0485 | .3399σ | .0490 ± .0185 |
| 2 and 2, 3 | .3572 ± .0380 | .3724 ± .0375 | .0152 | .1618σ | .0111 ± .0091 |
| 2 and 2, 4 | .6258 ± .0265 | .6358 ± .0259 | .0100 | .1725σ | .0126 ± .0097 |
| 2 and 3, 4 | .4269 ± .0356 | .5018 ± .0326 | .0749 | .4052σ | .0690 ± .0217 |
| 3 and 4 | .7410 ± .0196 | .7483 ± .0192 | .0073 | .1782σ | .0109 ± .0089 |
| 3 and 1, 2 | .4421 ± .0350 | .4608 ± .0343 | .0187 | .2220σ | .0169 ± .0112 |
| 3 and 1, 3 | .7073 ± .0218 | .7161 ± .0212 | .0089 | .1912σ | .0125 ± .0097 |
| 3 and 1, 4 | .5730 ± .0292 | .6000 ± .0279 | .0270 | .3040σ | .0317 ± .0152 |
| 3 and 2, 3 | .3285 ± .0388 | .3618 ± .0378 | .0333 | .2493σ | .0230 ± .0124 |
| 3 and 2, 4 | .4472 ± .0348 | .4632 ± .0342 | .0160 | .2063σ | .0146 ± .0104 |
| 3 and 3, 4 | .5336 ± .0311 | .5584 ± .0291 | .0247 | .2813σ | .0271 ± .0139 |
| 4 and 1, 2 | .3634 ± .0378 | .3966 ± .0367 | .0332 | .2229σ | .0252 ± .0135 |
| 4 and 1, 3 | .5640 ± .0297 | .5857 ± .0286 | .0217 | .2873σ | .0249 ± .0135 |
| 4 and 1, 4 | .6202 ± .0268 | .6459 ± .0249 | .0257 | .2533σ | .0325 ± .0157 |
| 4 and 2, 3 | .1374 ± .0427 | .1846 ± .0420 | .0471 | .1730σ | .0152 ± .0106 |
| 4 and 2, 4 | .4907 ± .0330 | .5133 ± .0321 | .0226 | .2114σ | .0227 ± .0129 |
| 4 and 3, 4 | .4489 ± .0348 | .4837 ± .0333 | .0348 | .2530σ | .0325 ± .0153 |

It will be remembered that a regression to be linear must have the constants $\eta - r$, $\eta^2 - r^2$ and $\Sigma_m$ equal to zero within the limits of random

sampling. To obtain these, two new constants must be derived. The correlation ratio is obtained by the formula

$$\eta = \frac{\sigma_{my}}{\sigma_y}$$

where $\sigma_{my}$ is the standard deviation of the weighted means of the $y$ arrays about the mean of the population. $\Sigma_m$, the square root of the mean square deviation of the means of the arrays from the regression line, is derived by the formula

$$\Sigma_m = \sigma_y \sqrt{\eta^2 - r^2}$$

due to PEARSON (1905). The probable errors of $\eta^2 - r^2$ are calculated by the method of BLAKEMAN (1905).

The net result of the study of table 21 shows that all the tables on which our correlations are based have the regression lines linear. In no case is the value of the constant $\eta^2 - r$ greater than three times its probable error.

The point of linearity of regression established, we may now return to table 20. From these constants the singular partial correlation coefficients where the total offspring output per female is held constant, have been tabulated in table 22.

TABLE 22

*Partial correlation coefficients for total single and total double crossing over in the third chromosome.*

| Doubles | Singles: Region 1 correlation Partial correlations | Region 2 correlation Partial correlations | Region 3 correlation, Partial correlation | Region 4 correlation, Partial correlation |
|---|---|---|---|---|
| 1, 2 .T | 0.4539 ± .0346 | 0.5471 ± .0305 | 0.2365 ± .0411 | 0.0560 ± .0434 |
| 1, 3 .T | 0.5895 ± .0284 | 0.3640 ± .0378 | 0.4423 ± .0350 | 0.0564 ± .0434 |
| 1, 4 .T | 0.3136 ± .0393 | 0.1158 ± .0430 | 0.1271 ± .0429 | 0.1966 ± .0419 |
| 2, 3 .T | 0.0676 ± .0433 | 0.3286 ± .0388 | 0.3352 ± .0386 | —0.0313 ± .0435 |
| 2, 4 .T | 0.2576 ± .0406 | 0.5077 ± .0323 | 0.1860 ± .0420 | 0.2714 ± .0403 |
| 3, 4 .T | 0.2062 ± .0417 | 0.2501 ± .0408 | 0.4200 ± .0359 | 0.2547 ± .0407 |

Study of this table reveals a general tendency on the part of crossing over in region 1, when correlated with doubles including region 1, to rise to a high point and then to decline from this point to the end of the chromosome. The same general tendency will be seen when region 4 is correlated with the successive doubles including 4. That is, the correlation rises to 2, 4, then declines toward 1, 4. In the middle of the chromosome both regions have their high correlations at the end of region 1. Thus it

TABLE 23

*Singular partial correlation coefficients for table 22 (second order).*

| Double | Singles, region 1 correlation | Double | Singles, region 2 correlation | Double | Singles, region 3 correlation | Double | Singles, region 4 correlation |
|---|---|---|---|---|---|---|---|
| 1, 2 . T2 | 0.3054 ± .0395 | 1, 2 . T1 | 0.4469 ± .0348 | 1, 2 . T1 | 0.0934 ± .0432 | 1, 2 . T1 | 0.0072 ± .0435 |
| 1, 2 . T3 | 0.4078 ± .0362 | 1, 2 . T3 | 0.5137 ± .0320 | 1, 2 . T2 | 0.0914 ± .0432 | 1, 2 . T2 | 0.0370 ± .0435 |
| 1, 2 . T4 | 0.4152 ± .0347 | 1, 2 . T4 | 0.5459 ± .0306 | 1, 2 . T4 | 0.2351 ± .0411 | 1, 2 . T3 | 0.0494 ± .0434 |
| 1, 3 . T2 | 0.5190 ± .0318 | 1, 3 . T1 | 0.1719 ± .0423 | 1, 3 . T1 | 0.3124 ± .0393 | 1, 3 . T1 | —0.0099 ± .0435 |
| 1, 3 . T3 | 0.5170 ± .0319 | 1, 3 . T3 | 0.2709 ± .0403 | 1, 3 . T2 | 0.3752 ± .0374 | 1, 3 . T2 | 0.0427 ± .0435 |
| 1, 3 . T4 | 0.5872 ± .0285 | 1, 3 . T4 | 0.3624 ± .0378 | 1, 3 . T4 | 0.4413 ± .0351 | 1, 3 . T3 | 0.0461 ± .0434 |
| 1, 4 . T2 | 0.2936 ± .0398 | 1, 4 . T1 | —0.0118 ± .0435 | 1, 4 . T1 | 0.0114 ± .0435 | 1, 4 . T1 | 0.1720 ± .0423 |
| 1, 4 . T3 | 0.2902 ± .0399 | 1, 4 . T3 | 0.0827 ± .0433 | 1, 4 . T2 | 0.0958 ± .0431 | 1, 4 . T2 | 0.1928 ± .0419 |
| 1, 4 . T4 | 0.2997 ± .0396 | 1, 4 . T4 | 0.1090 ± .0435 | 1, 4 . T4 | 0.1211 ± .0429 | 1, 4 . T3 | 0.1940 ± .0419 |
| 2, 3 . T2 | —0.0745 ± .0433 | 2, 3 . T1 | 0.3299 ± .0388 | 2, 3 . T1 | 0.3365 ± .0386 | 2, 3 . T1 | 0.0241 ± .0435 |
| 2, 3 . T3 | —0.0572 ± .0434 | 2, 3 . T3 | 0.2533 ± .0408 | 2, 3 . T2 | 0.2662 ± .0406 | 2, 3 . T2 | 0.0173 ± .0435 |
| 2, 3 . T4 | 0.0646 ± .0434 | 2, 3 . T4 | 0.3276 ± .0389 | 2, 3 . T4 | 0.3375 ± .0386 | 2, 3 . T3 | 0.0210 ± .0435 |
| 2, 4 . T2 | 0.0679 ± .0433 | 2, 4 . T1 | 0.4568 ± .0344 | 2, 4 . T1 | 0.1061 ± .0430 | 2, 4 . T1 | 0.2533 ± .0407 |
| 2, 4 . T3 | 0.2092 ± .0416 | 2, 4 . T3 | 0.4821 ± .0334 | 2, 4 . T2 | 0.0417 ± .0435 | 2, 4 . T2 | 0.2883 ± .0399 |
| 2, 4 . T4 | 0.2382 ± .0411 | 2, 4 . T4 | 0.5151 ± .0320 | 2, 4 . T4 | 0.1837 ± .0421 | 2, 4 . T3 | 0.2699 ± .0404 |
| 3, 4 . T2 | 0.1192 ± .0429 | 3, 4 . T1 | 0.1867 ± .0420 | 3, 4 . T1 | 0.3795 ± .0373 | 3, 4 . T1 | 0.2387 ± .0411 |
| 3, 4 . T3 | 0.0699 ± .0433 | 3, 4 . T3 | 0.1439 ± .0426 | 3, 4 . T2 | 0.3737 ± .0374 | 3, 4 . T2 | 0.2515 ± .0408 |
| 3, 4 . T4 | 0.1855 ± .0420 | 3, 4 . T4 | 0.2468 ± .0409 | 3, 4 . T4 | 0.4256 ± .0356 | 3, 4 . T3 | 0.2651 ± .0405 |

is seen that the relation of double crossovers to the regions surrounding them forms curves, the crest of the curves occurring between 20 and 30 units.

But there are discrepancies in the high point of this curve. It will be noticed that there is a significant correlation between region 1 and the double including regions 2 and 4. In the same way, 2 is correlated with 1, 3 and 3 with 1, 4 significantly as measured by its probable error. These correlations would not be expected, and the question arises as to what they are due.

We have seen that the single crossovers in the various regions are correlated variates in which the correlation is most pronounced between adjacent regions. If, then, crossing over in region 1 has a sufficiently high correlation with crossing over in region 2, it would be expected that double crossing over including region 2 would also be correlated with region 1. To test this hypothesis for this case and the similar cases as given above, it is necessary to form the previously described universe, in which the correlation between the two continuous variables 1 and 2, 4 for a constant value of a third variable 1 and 2 is determined, where all values of the offspring are held constant. The measure of such a correlation has been termed the singular partial correlation by PEARSON (1914).

The values for the successive singular partial correlation coefficients (second order) for the above data are given in the table below in which the terms held constant are separated from the correlated term by a dot.

For the complete analysis of the problem the third-order singular partial coefficients needed are given in table 24.

TABLE 24

*Singular partial correlations for table 23 (third order).*

| Double | Singles region 1 | Double | Singles region 2 |
|---|---|---|---|
| 2, 3 .2T3 | —0.0845 ± .0432 | 1, 3 .1T3 | 0.1223 ± .0429 |
| 2, 4 .2T4 | 0.0421 ± .0435 | 1, 4 .1T4 | —0.0123 ± .0435 |
| 3, 4 .3T4 | 0.0442 ± .0435 | 3, 4 .3T4 | 0.1834 ± .0421 |

| Double | Singles region 3 | Double | Singles region 4 |
|---|---|---|---|
| 1, 2 .1T2 | 0.0124 ± .0435 | 1, 2 .1T2 | 0.0070 ± .0435 |
| 1, 4 .1T4 | 0.0124 ± .0435 | 1, 3 .1T3 | 0.0119 ± .0435 |
| 2, 4 .2T4 | 0.0371 ± .0435 | 1, 4 .1T4 | 0.0169 ± .0435 |

A study of these tables shows that where before a significant correlation was observed between singles in one region and doubles not including this region as one of the breaking segments when coefficients of the first order were used, now a significant correlation is present only when the double also includes the single as one of the breaking segments. Thus the previous hypothesis to account for these discrepancies was correct. Two double crossovers correlated with a given region may have a significant correlation with that region due to their both being correlated with a third region.

The last difficulty in the study of the data at hand for the purpose of determining how double crossovers are related to the various regions of the chromosome is removed. The correlations of table 23 show that double crossing over, including region 1, is not distributed at random, but is more apt to have a second simultaneous break in region 3 than in any other, the difference in this case running as high as seventeen times the probable error.

Each of the end regions exhibit that rise in the relationship in the middle of the chromosome which, as has been previously pointed out in the first part of the paper, would be expected on the basis of the twisting hypothesis where there was a definite ratio of twist. These waves rise rather sharply to the mid-point and drop off rapidly in the other direction. The relations of region 1 to double crossing over including the other successive regions is that best suited to bring out this rise, for, as has been previously pointed out, the region 1 is so short that twists of the same length cannot extend into either of two regions. This, then, forms the best test of the hypothesis of twisting to account for crossing over versus any other hypothesis which calls for the distribution of crossovers at random. Region 1 correlated with the successive doubles rises sharply to 3 and falls rapidly to 4. Region 4 rises to 2 and falls to 1. The rise and fall in 4 is less rapid, due to its being so long a segment that it enables a twist falling within its bounds to fall in either of two regions, depending on whether or not its first break is near one end or the other, still even in this the mode is marked. The mid-regions also exhibit a rise toward the ends as would be expected, although as the number of factors is too few, no mode appears. This high point and this drop may then represent a twisting taking place about every 20 to 30 units in the third chromosome.

If these correlations are considered from the point of view of the reduplication theory to account for the interchange, what is it necessary to consider? Not only does the rise and fall of the correlation have to be

taken into account but also account has to be taken of the whole correlation, for no correlation can be expected on the reduplication theory as previously shown. Correlations have then to consider nothing, even when ranging from twenty-four times the probable error to those four times the probable error. The odds against this being accidental are enormous. It may then be said that the twisting hypothesis for crossing over accounts for the facts remarkably well, while the reduplication theory accounts for them not at all. Consequently, the experimental facts here deduced in carefully controlled and analyzed experiments indicate crossing over to take place between loosely twisted, finely spun-out chromosome threads with between 25 and 30 units as the modal distance between successive crossovers. Because of the uneven intervals and the inaccuracy of the moments calculated from them due to the few classes on which they would have to be based, it is not the purpose of this paper to treat the frequencies other than by the use of correlation coefficients. It is, however, of interest to try the moment calculation for the position of mode for the best suited of these classes for analysis. The approximate ratio between the two twists when calculated for the $s_eD'$ region by the formula: position of the mode $=$ mean $- \frac{1}{2} \frac{1\mu_3(r-2)15}{2\mu_2(4+2)}$, is shown to be at 26.24 units, considering each class as distributed around the mid-ordinates (an hypothesis obviously untrue, but giving the best approximation to the true value which it is possible to obtain since the true mean of each class is not known). The use of the above formula for the mean is justified, as the curve is shown to be type IV by $\beta_1 = +.057$ and $\beta_2 = +68.313$.

## DISCUSSION

The geometrical interpretation put upon the rise and fall of the double-crossover frequencies may seem rather speculative in character. It is, however, I venture to think, supported by a good deal of strong evidence. Since the idea that the chromosomes are the bearers of the determiners of hereditary characters was put forward by WEISMANN and ROUX and applied to Mendelian inheritance by SUTTON, there has been an ever increasing amount of evidence collected that it is to the chromosome we must look for the mechanism of heredity. As a basis for this conclusion the studies of STEVENS and WILSON have shown the parallel between sex and the behavior of a chromosome pair. This was followed by the work of MORGAN, showing that this parallel included the so-called sex-linked factors as well as sex. As direct evidence, we may draw first on that of BOVERI on multipolar mitosis, of BALTZER on reciprocal crosses of sea-

urchins and that of HERBST (1909) and GODLEWSKI (1911) on parthenogenesis and fertilization. Further evidence comes from the work of LUTZ (1912), GEERTS (1911) and GATES (1907, *et seq.*) in the study of Oenothera mutants. With all, perhaps the most brilliant piece of evidence is that of BRIDGES (1916) where proof is given that the sex chromosomes bear the sex-linked factors; for here by cytology and genetics he can follow the course of the sex-chromosomes and of the factors carried thereby. These names do not exhaust the list of those who have added materially to the proof that the chromosomes are the bearers of the hereditary characters, yet it seems to me that these constitute as complete a chain of crucial experimental evidence as would be required by the most rigorous logic, consequently the conclusion will not be crowded by presenting more. Should it be granted, however, that this does constitute proof, it requires that all hypotheses to account for the interchange of linked or coupled factors shall rest on the chromosome; it requires that the reduplication hypothesis shall segregate its genetic ratios through the agency of the chromosomes.

If other evidence is taken purely from the experimental side of genetics and consideration be given to STURTEVANT'S (1914) criticism, the reduplication hypothesis in what TROW (1912) and BAILEY (1913) have shown to be the general mathematical relations of its gametes, it is found that it is doubtful if reduplication is able to explain even the ratios that are obtained. Thus in the case of TROW'S special hypothesis STURTEVANT shows that in every case the calculated is greater than the observed ratios. The difference is significant in every case and in the same direction. Further, STURTEVANT shows that if the general hypothesis is used the number of cell divisions required are at hopeless variance when considered with the possible divisions. It may then be said that deduction from the theory leads to a poor agreement between this theory and fact.

The students of genetics who use the linkage hypothesis to explain their ratios have some evidence to show that the linkage hypothesis is also applicable to the same forms on which the reduplication hypothesis is based. BRIDGES (1914) has shown that in the experiments of PUNNETT (1913) on sweet peas and GREGORY (1911) on Primula the linkage hypothesis is at least applicable. Unfortunately, BRIDGES used as his measure of linkage the coefficient of association of YULE, which is in itself of rather doubtful value as a measure of relationship, as has been shown by HERON (1911), and HERON and PEARSON (1913). Fortunately, however, the conclusions of BRIDGES have been justified, since we now have some very excellent data presented by ALTENBURG (1916)

in evidence that the linkage hypothesis can be applied to Primula. Data on some 3600 plants show clearly that it is exceedingly difficult to see how a reduplicating series can be made to fit.

ALTENBURG separates his cases in which the male plants were heterozygous and the cases in which his female plants were heterozygous. There seemed to be quite a difference in the amount of crossing over between them, so I thought it might pay to test each of the classes for the likelihood of this coming from random sampling. The table 25 shows the result of this test.

TABLE 25

| | ♂ Heterozygous | ♀ Heterozygous | Percent ♂ | Percent ♀ | Difference | Diff. ÷ P.E. Diff. |
|---|---|---|---|---|---|---|
| Non-crossovers | 1829 | 266 | 55.558929 | 67.807132 | 12.298 ± 1.716 | 7.2 |
| First single | 1053 | 107 | 31.986633 | 27.295914 | 4.691 ± 1.437 | 3.2 |
| Second single | 325 | 11 | 9.872418 | 2.806122 | 7.066 ± 0.956 | 7.4 |
| Double | 85* | 8* | | | | |

* Not calculated, as the value of $q$ would be very small.

Thus it is seen that the difference is well above three times the probable error. The range of probability that these differences came from random sampling are 31 to 1 for the 3.2 times the probable error to 1,675,321 to 1 for the 7.2 times the probable error. This difference certainly looks significant. Since all of these plants were raised under the same conditions and cared for alike, it would seem that crossing over in the female is less than that in the male due to some differential effect of the sex. Such a graded effect, taken in connection with the other known facts for crossing over, indicates that when a sufficient number of animals and plants are known, a graded series of crossing-over values may be found, extending from Drosophila with crossing over only in the female, through sweet pea and Primula with crossing over in both sexes, to silk-worms and probably chickens, with crossing over only in the male. Such a series would then duplicate the series found for the Y chromosomes, although it would not parallel it.

To return to our general theme, in discussing this paper of ALTENBURG (1916), PUNNETT (1917) suggests that the reduplication hypothesis calls for a marked difference for the reduplicating series for the *BC* regions in the back crosses, $\frac{BAC}{bac} \times \frac{bac}{bac}$ and $\frac{BC}{bc} \times \frac{bc}{bc}$.[4]

[4] "On the chromosome hypothesis there is only one set of positions which allows of

At first sight the difference in crossing over shown when different genes are interpolated between two fixed genes would seem to agree with this expectation for the reduplication hypothesis as above stated. In non-conformity with the requirements of this hypothesis as stated by PUNNETT, the total crossing over may be significantly increased by the presence of *A* heterozygous and be diminished in a like case where another *A* is heterozygous as compared with the crossing over of the $\frac{BC}{bc} \times \frac{bc}{bc}$ condition. Further, the change of a factor outside the region *BC* may affect the crossing over of that region more than a similar change of a factor within the region itself. Let us now take the data in the tables and arrange two cases to conform with the needs of the reduplication hypothesis. There are several possible cases of this kind which could be made. The choice of the particular case seems immaterial, consequently let us confine attention to the back-crossed females heterozygous for sepia, spineless on one side, dichaete on the other, and females heterozygous for the genes sepia and spineless. For this table the data from appendix tables A, D, and C are available. The data collected and so reduced are given in tables 26 and 27.

two of the coupling values between three factors *A*, *B* and *C* to be equal, viz., when two loci are equidistant from the third, thus:

*B* *A* *C*

The coupling or linkage values between *A* and *B*, and between *A* and *C*, are here of the same value, but when this is so it follows of necessity that the value for *B* and *C* must be considerably lower than either of the other two. If a three-factor case were found of such a nature that two of the values were equal and the third definitely higher, such a case might serve as a criterion between the two hypotheses." Further, he says, "Such a case is probably to be found among Primulas in connection with the three pairs of characters, magenta (*M*) and red (*r*), short style (*S*) and long style (*s*), green stigma (*G*) and red stigma (*g*)." GREGORY and ALTENBURG have both published on these, but, as PUNNETT, says, "The figures" (ALTENBURG's) "as they stand offer of course no criterion between the rival hypotheses, for the critical experiment is yet to be made. This consists in the cross between *SsGg* plants (ex *SG* × *sg*) and the double recessive *ssgg*, where all individuals used are homozygous for either *M* or *m*. On the chromosome hypothesis the linkage values should remain the same as those given above (where *M* is present in heterozygous forms); on the reduplication hypothesis we should expect to find the linkage higher, probably of the form *2SG*: *1Sg*: *1sG*: *2sg*." This statement has several obscure points more especially as to how this comparison of the two distributions is to be made. If, as the text would indicate, the comparison is to be between the reduplication series as applied to each set of data, instead of comparing the actual distribution of the data, the reasoning is in error for, as STURTEVANT has shown that the series obtained on TROW's hypothesis, always is significantly too high. In our comparison we shall, therefore, consider only the actual figures.

TABLE 26

| | $D'$ | $s_c\ s_s$ | $s_cD'\ s_s$ | $s_s$ | $D's_s$ | $s_c$ | $s_cD's_s$ | $N$ |
|---|---|---|---|---|---|---|---|---|
| Table A | 3532 | 3257 | 381 | 368 | 244 | 307 | 27 | 26 |
| Table D | 12758 | 12357 | 1666 | 1583 | 1260 | 1507 | 145 | 196 |

For table C when dichaete is not present:

TABLE 27

| | Non-crossovers | | Single crossovers | |
|---|---|---|---|---|
| | $s_cs_s$ | $N$ | $s_c$ | $s_s$ |
| Table C | 568 | 1153 | 277 | 233 |

Considering only the series $s_cs_s$ which, according to PUNNETT's statement of the reduplication hypothesis, the series of A and D should differ from that of C we have:

TABLE 28

| | $s_c\ s_s$ | $s_c$ | $s_s$ | $N$ |
|---|---|---|---|---|
| Table A ..... | 3284 | 688 | 612 | 3558 |
| Best fitting series 5:1:1:5 | *3395* | *679* | *679* | *3395* |
| Table D ..... | 12502 | 3173 | 2843 | 12954 |
| Best fitting series 4:1:1:4 | *12589* | *3147* | *3147* | *12589* |
| Table C ..... | 968 | 277 | 233 | 1153 |
| Best fitting series 4:1:1:4 | *1052* | *263* | *263* | *1052* |

None of these expected series agrees with the actual series as well as could be wished. Thus table A could be best fitted by a series of about 4.6 to 1. Table C would have a better agreement between actual and expected, fitted with a series of 4.4 to 1. This is a fundamental drawback to the theory of reduplication, for the search for simple series often obscures real differences. Thus the Primula series treated by PUNNETT (1917) leads to a theoretical distribution on TROW's hypothesis of secondary reduplication which could have the actual distribution observed in the *SG* series selected from it in samples of 3684 individuals each in not more than 1 in 2500 such samples. Yet the uncritical nature of the hypothesis led PUNNETT to conclude that the result is in fair accord with expectation. Thus in our experiment there are significant differences in the distributions taken as a whole, but these differences follow no rule.

Comparing the reduplicating series above, it is seen that they lead to practically the same thing; that is, 4:1:1:4, instead of coming to markedly different series as the reduplication hypothesis calls for. The conclusion seems forced upon us by this test of the hypothesis that the facts of the several factor crosses do not agree with this hypothesis, but that the twisting hypothesis, based as it is on the known chromosome behavior, does fit the facts.

The conclusion that the reduplication hypothesis does not explain the several factor cases is further borne out by the fundamental experiments of PLOUGH (1917). In this investigation the temperature effect on crossing-over rate enabled him to show that crossing over does not occur in the early oögonial divisions, and there is good reason to believe that the percentage of crossing over is affected by temperature only in the growth period of the egg. Thus the long series of differential cell divisions necessary for the formation of the reduplicating series is shown to be absent in actual point of fact.

Adopting the explanation that it is the chromosomes that must be looked to for the mechanism of crossing over, the inquiry may be made as to how crossing over is brought about. STURTEVANT (1913) has shown that it is possible to map the position of the factors in the chromosome by crossing-over ratios. This principle has been extended and has been shown to be applicable to all the Drosophila chromosomes. The practical value of the hypothesis may thus be said to be proved, and through the work of MORGAN, STURTEVANT, BRIDGES and MULLER (1915) all of the chromosomes are mapped. As was pointed out in a preceding section of this paper, the ratios may vary, yet in no case does this variation affect the relative position of the factors.

It becomes important, then, to inquire how this exchange takes place. The function of this paper is a specific inquiry into what the ratios of the double crossovers to the different single crossover regions would show as to this interchange. Following the ideas of JANSSENS (1909) as elaborated by MORGAN (1916), MULLER, BRIDGES, and PLOUGH (1917), of a twisting of the chromosomes, although considering that this twisting takes place at an earlier stage and between the finer threads of the chromosomes, it has been possible to show that the results are what would be expected on the twisting hypothesis to account for crossing over. Turning more to the general aspects of the case, it is seen that this gives a strong foundation for a single mechanical explanation for the exchange of factors where there is only one scheme to account for the whole. It is not known why a fusion should take place where it

does or why the genes should line up along the chromosomes as accurately as they do. These are problems of the future, but they do not in any way influence the fact that the hypothesis of loose twisting explains the observed ratios for the third chromosome in so many of its intricacies, as to carry the conviction that it is much more than just a chance relationship.

In conclusion, I wish to express my thanks to Prof. E. B. WILSON and Dr. C. B. DAVENPORT for opportunities accorded me for research; to Prof. T. H. MORGAN, Dr. A. H. STURTEVANT, and Dr. C. B. BRIDGES for their ever-ready assistance and suggestion, and to Dr. RAYMOND PEARL for his constant interest in my work.

## SUMMARY

This paper is a contribution toward the analysis of the normal fluctuating variations in crossing over as seen in the third chromosome of *Drosophila melanogaster*. The third chromosomes of each female, as shown by her offspring, cross over a certain number of times. The variability is studied by comparing the results from different females. The association between the crossovers is studied by comparing the results within each given female.

1. The means, standard deviations, and the coefficients of variation are given for the distributions of each region in this chromosome under discussion. The features of chief interest are the great variability of both single crossing over and double crossing over. The coefficient of variability ranges between 18 and 59 for the single crossovers and 67 and 110 for the double crossovers. The actual amount of this coefficient is apparently dependent to some degree on the actual mean size of the crossing-over ratio.

2. A table is presented to show the relative variability of crossing over in comparison with that of other physiological and morphological characters. The table shows that crossing over is one of the most highly variable phenomena known, indicating that the mechanism behind crossing over is not as precise as that found in most physiological studies.

3. As a necessary preface to the analysis of the internal mechanism of variations of crossing over it has been pointed out that it is essential to know how much the ratios are influenced by external agencies. Toward this end it has been shown (page 214) that to some degree the absolute value of the crossing-over ratio varies according to the genes present in the chromosome. Further, it is shown that no significant

effect on crossing over was produced by the food or temperature used or by the variations of season or bottle output.

4. Since it had been shown that the crossing-over values are influenced by the known genes present, it became essential to know whether or not there were any modifying genes present influencing the ratios. A selection experiment was performed to test this. The parent and offspring correlations for this experiment ranged from + 0.336 ± .135 to — .0211 ± .118 for the selection for low crossing over; for high crossing over from + 0.150 ± .248 to — 0.134 ± .191. The conclusion is to be drawn that there were no differences in modifying factors for crossing over in the experiment.

5. In the resolution of the single crossing-over ratios into their component elements it was shown that there is a significant correlation between the crossing over in different regions. In general this difference progresses from the left-hand end of the chromosome to the right. Thus the correlation between region 1 and 2 is + 0.4019 ± .0365, between 1 and 3 is + 0.3492 ± .0382, and between 1 and 4 is + 0.1039 ± .0430. The explanation of this difference is obscure.

6. A relationship between single and double crossing over is shown to exist, such that a crossover in one region is more likely to be accompanied by another simultaneous crossing over in a region 25 to 35 units away than it is to be accompanied by a simultaneous crossing over in any other region. Thus when region 1 is correlated with its double crossover including regions 2, 3, and 4, respectively, the correlations are + 0.3054 ± .0395, + 0.5170 ± .0319, and + 0.2997 ± .0396. This rise and fall, together with a definite mode, is held to mean that there is a modal interval between two successive crossovers. Thus the two finely spun-out chromosomes, when they come together prior to crossing over, apparently twist about each other loosely and generally have the points of contact where breaking may take place about 25 to 30 units apart.

## APPENDIX

### TABLE B

$$Cross: \varphi \frac{D' \quad p^p \quad s_s \quad c^s \quad r_o}{N} \times \delta \frac{p^p \quad s_s \quad c^s \quad r_o}{p^p \quad s_s \quad c^s \quad r_o}$$

| References | 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | 1,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 627 | 46 59 | 3 3 | 16 12 | 17 14 | 19 9 | 1 1 | . 2 | 2 2 | 1 1 | 2 3 | . 1 | . . | . . | . . | 214 |
| 629 | 45 41 | 9 9 | 8 12 | 13 15 | 17 6 | 1 1 | . . | 3 1 | . . | 2 2 | . . | . . | 1 . | . . | 186 |
| 632 | 29 34 | 2 8 | 17 9 | 15 3 | 11 7 | . . | 1 . | 2 1 | 2 1 | 2 1 | . 3 | . . | . 1 | . . | 149 |
| 634 | 47 57 | 2 2 | 14 15 | 15 18 | 19 20 | . 3 | 1 2 | 1 1 | . 1 | . 2 | . . | . . | . . | . . | 220 |
| 658 | 42 45 | 1 1 | 4 3 | 4 11 | 11 9 | . . | 1 2 | 1 . | . . | 1 2 | . . | . . | . . | . . | 138 |
| 665 | 78 90 | 6 9 | 24 21 | 24 27 | 21 26 | . 2 | 3 4 | 3 . | . . | 4 5 | 1 2 | 1 . | . 2 | . . | 353 |
| 665b | 51 64 | 2 5 | 13 15 | 16 11 | 17 16 | 1 . | 1 1 | . . | 1 . | 1 . | 1 . | . . | . . | . . | 216 |
| 665c | 42 52 | . 5 | 2 10 | 7 8 | 24 11 | . . | 1 2 | 3 . | . . | 1 . | . . | . . | . . | . . | 168 |
| 700c | 22 27 | 1 1 | 4 3 | 4 3 | 9 5 | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | 80 |
| 706 | 25 18 | 2 . | 2 1 | 4 7 | 6 8 | . . | . . | . . | . . | . . | 1 . | . . | . . | . . | 74 |
| 708a | 23 31 | 5 2 | 9 6 | 11 10 | 9 7 | 1 2 | 1 1 | . . | . 2 | . 1 | . 2 | . . | . . | . . | 123 |
| 708b | 15 25 | 2 1 | 4 3 | 5 5 | 5 6 | . . | . . | . . | . . | 1 . | . . | . . | . . | . . | 71 |
| 708c | 27 39 | 2 1 | 5 8 | 7 7 | 10 10 | . . | . . | . . | . . | 1 . | . . | . . | . . | . . | 117 |
| 710 | 15 22 | . . | 2 . | 4 4 | 14 4 | . . | . . | . . | . . | . 1 | . . | . . | . . | . . | 66 |
| 718b | 23 34 | 1 2 | 8 8 | 6 9 | 8 5 | . 1 | . . | 1 4 | . . | . . | . . | . . | . . | 1 . | 111 |
| 728a | 25 27 | 4 1 | 2 3 | 3 5 | 12 5 | . 1 | . 2 | 2 2 | . . | . 1 | . . | . . | . . | . . | 95 |
| Total | 1220 | 92 | 263 | 312 | 366 | 15 | 25 | 30 | 9 | 32 | 11 | 1 | 4 | 1 | 2381 |

### TABLE C

$$Cross: \varphi \frac{s_o \quad s_s \quad c^s \quad r_o}{H'} \times \delta \frac{s_o \quad s_s \quad c^s \quad r_o}{s_o \quad s_s \quad c^s \quad r_o}$$

| References | 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1052 | 42 20 | 8 15 | 3 6 | . 1 | 7 6 | 2 2 | . . | 2 1 | . . | . . | . . | . . | . . | 115 |
| 1157 | 56 55 | 6 13 | 5 10 | . . | 9 16 | . . | 1 . | 1 5 | 1 2 | . . | 2 . | 1 . | . . | 183 |
| 1161 | 55 45 | 7 7 | . 3 | . . | 20 21 | . 1 | . . | 1 6 | . . | 1 1 | . . | . . | . . | 168 |
| 1162 | 57 51 | 20 12 | 10 6 | 1 . | 18 18 | 1 3 | . . | 1 1 | . . | . . | . . | . . | . . | 199 |
| 1163 | 54 40 | 9 9 | 2 6 | . . | 6 7 | . . | . 1 | 2 . | . . | . . | . . | . . | . . | 136 |
| 1189 | 60 58 | 18 14 | 10 5 | . 3 | 19 18 | 1 . | . . | 4 7 | . . | . . | . . | . . | . . | 217 |
| 1192 | 38 28 | 14 8 | 4 2 | 1 . | 12 13 | 1 . | . . | 5 5 | 4 . | . . | . . | 2 . | . . | 137 |
| 1193 | 58 66 | 21 18 | 7 14 | . 2 | 25 17 | 1 2 | . . | 3 8 | . 1 | 1 . | . . | . . | . . | 244 |
| 1194 | 39 31 | 13 13 | 2 4 | 1 1 | 8 12 | . . | . . | 2 5 | . . | . 1 | . . | . . | . . | 132 |
| 1195 | 66 40 | 12 10 | 4 15 | . . | 23 24 | 3 1 | 3 1 | 5 1 | . . | 2 1 | . . | . . | 1 . | 212 |
| 1197 | 87 58 | 21 11 | 9 9 | 1 1 | 24 18 | . . | . . | 2 3 | 1 . | 2 . | . . | . . | . . | 247 |
| 1229 | 17 20 | 7 2 | 2 1 | 1 . | 2 2 | 2 . | . . | 1 1 | . . | . . | . . | . . | . . | 58 |
| 1242 | 18 16 | 5 2 | . 1 | . . | 2 3 | . 1 | . . | . . | . . | . . | . . | . . | . . | 48 |
| 1249 | 39 27 | 13 9 | 4 4 | . 1 | 10 15 | . 1 | . . | 3 1 | . . | . . | . . | . . | . . | 127 |
| 1250 | 38 29 | 8 9 | . 5 | . 1 | 7 2 | 3 2 | . . | 2 . | . . | 1 . | . . | . . | . . | 107 |
| 1251 | 27 21 | 12 8 | 5 2 | 2 1 | 7 6 | . . | . . | 3 1 | . . | . . | . . | . . | . . | 95 |
| 1270 | 12 12 | 1 . | 1 3 | . 1 | 8 5 | 1 . | . . | 3 . | . 1 | . . | . . | . . | . . | 48 |
| 1273 | 15 15 | 7 6 | 2 2 | . 1 | 4 6 | . 1 | . . | . 2 | . . | . . | . . | . . | . . | 61 |
| 1280 | 15 13 | 1 3 | 2 3 | . . | 9 7 | . 1 | . 2 | . . | . . | . . | . . | . . | . . | 56 |
| 1275 | 10 11 | 2 4 | 1 1 | . 2 | 6 1 | 1 . | . . | . 2 | . . | . . | . . | . . | . . | 41 |
| Total | 1459 | 378 | 175 | 22 | 443 | 31 | 8 | 89 | 10 | 10 | 2 | 3 | 1 | 2631 |

TABLE A

*Cross:* ♀ $\frac{Sa\ Cu\ Sa\ e^{s}\ r_{o}}{D'\qquad H'} \times ♂ \frac{Sa\ Cu\ Sa\ e^{s}\ r_{o}}{Sa\ Cu\ Sa\ e^{s}\ r_{o}}$

| References | 0 | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 1, 2 | | 1, 3 | | 1, 4 | | 1, 5 | | 1, 6 | | 2, 3 | | 2, 4 | | 2, 5 | | 2, 6 | | 3, 4 | | 3, 5 | | 3, 6 | | 4, 6 | | 1,2,3 | | 1,2,5 | | 1,2,6 | | 1,3,4 | | 1,3,6 | | 1,4,6 | | 2,3,6 | | 3,4,6 | | 3,5,6 | | Total |
|---|---|---

TABLE D

Cross: $\varphi \frac{s_o \; c^a \; r_o}{D'} \times \delta \frac{s_o \; s_s \; c^b \; r_o}{s_o \; s_s \; c^b \; r_o}$

| References | 0 | | 1 | | 2 | | 3 | | 4 | 1,2 | 1,3 | 1,4 | 2,3 | 2,4 | 3,4 | 1,2,3 | 1,2,4 | 1,3,4 | 2,3,4 | 1,2,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 588 | 27 | 25 | 5 | 5 | 6 | 3 | 10 | 4 | 8 | 2 | . | . | . | I | . | . | . | . | . | . | 97 |
| 666 | 14 | 14 | 2 | 2 | 3 | 4 | 4 | 5 | 2 | . | 3 | . | I | . | . | . | I | . | 4 | . | 60 |
| 737 | 8 | 12 | 3 | I | I | I | 2 | 5 | 8 | 2 | . | . | . | I | . | . | . | . | . | . | 44 |
| 740 | 53 | 63 | 7 | 14 | 10 | 10 | 6 | 17 | 26 | 28 | 3 | 2 | I | 3 | 3 | 3 | I | . | I | 2 | 244 |
| 741 | 4 | 6 | . | I | . | I | I | . | I | 3 | . | . | . | . | I | . | . | . | I | . | 19 |
| 747 | 53 | 47 | 8 | 10 | 9 | 9 | 6 | 6 | 23 | 25 | . | I | 4 | 3 | . | I | . | . | I | 5 | 213 |
| 748 | 11 | 4 | I | 2 | I | 2 | I | 3 | 4 | 4 | I | . | . | . | . | I | . | . | . | I | 36 |
| 750 | 43 | 36 | 6 | 4 | 5 | 3 | 2 | 7 | 16 | 7 | . | I | 2 | . | I | 2 | . | . | I | 2 | 139 |
| 751 | 55 | 20 | . | I | 2 | . | 2 | 4 | 8 | 6 | . | . | . | . | 2 | . | I | I | . | . | 102 |
| 754 | 74 | 66 | 3 | 2 | I | 2 | 3 | . | 6 | I | . | . | . | . | . | . | . | . | I | . | 45 |
| 756 | 15 | 11 | 4 | 7 | 3 | 8 | 10 | 7 | 28 | 24 | . | . | I | . | I | I | I | . | I | . | 238 |
| 757 | 26 | 29 | 4 | 2 | 4 | 6 | 2 | 4 | 9 | 6 | . | . | . | I | I | . | . | . | . | . | 94 |
| 758 | 6 | 6 | 2 | . | I | . | 2 | I | I | . | . | . | . | I | . | . | . | . | . | . | 20 |
| 759 | 63 | 63 | 6 | 6 | I | 6 | 7 | 6 | 25 | 29 | . | 2 | . | 4 | 2 | 3 | I | . | I | 2 | 228 |
| 760 | 44 | 49 | 5 | 3 | 2 | 7 | 4 | 5 | 17 | 9 | . | I | I | 2 | . | 3 | . | . | I | . | 156 |
| 761 | 41 | 41 | 5 | 7 | . | 2 | . | 6 | 22 | 20 | . | I | . | . | . | 2 | . | . | . | . | 149 |
| 762 | 60 | 57 | 6 | 7 | 2 | 4 | 9 | 5 | 20 | 18 | I | . | . | . | 2 | . | . | I | I | . | 193 |
| 763 | 52 | 53 | 0 | 5 | 3 | 4 | 10 | 7 | 14 | 12 | I | I | I | . | 3 | 4 | I | . | I | . | 171 |
| 764 | 40 | 35 | 5 | 3 | 4 | I | 8 | 5 | 6 | 12 | I | . | I | . | 3 | . | . | . | 2 | . | 125 |
| 766 | 38 | 40 | 4 | 4 | . | 6 | 3 | 3 | 12 | 10 | I | 2 | . | 3 | 2 | 2 | . | . | I | I | 132 |
| 767 | 27 | 35 | 4 | 5 | 5 | 5 | 3 | 2 | 16 | 7 | . | . | . | . | . | . | . | . | . | . | 109 |
| 770 | 14 | 19 | 2 | 2 | I | 3 | I | 2 | 6 | . | . | . | I | I | . | . | . | . | . | . | 52 |
| 772 | 9 | 10 | I | I | I | . | 2 | . | . | I | . | . | . | . | . | . | . | . | I | . | 26 |
| 774 | 28 | 18 | 2 | 3 | I | 2 | I | 4 | 10 | 9 | . | . | . | I | . | 3 | . | . | I | . | 83 |
| 775 | 52 | 20 | 4 | 7 | I | 2 | I | 3 | 2 | 10 | . | I | . | I | I | . | . | . | . | . | 105 |
| 776 | 38 | 31 | 2 | 3 | 7 | 9 | 5 | 4 | 5 | 12 | 2 | I | 2 | I | . | I | . | . | I | I | 127 |
| 777 | 22 | 24 | 11 | 2 | I | 3 | 7 | 2 | 14 | 8 | . | . | . | . | I | I | . | I | I | . | 99 |
| 778 | 25 | 20 | 2 | 4 | 7 | 9 | 4 | 4 | 8 | 18 | . | 3 | 2 | I | 3 | 2 | . | . | 2 | 2 | 116 |
| 779 | 40 | 48 | I | I | 6 | 6 | 8 | 7 | 15 | 18 | . | . | . | . | I | 4 | 2 | I | I | . | 160 |
| 780 | 31 | 31 | 2 | 2 | 3 | 4 | 4 | 5 | 4 | 4 | . | . | . | . | . | . | . | . | I | . | 91 |
| 781 | 27 | 28 | . | 2 | 9 | I | 4 | 6 | 14 | 13 | I | . | . | . | . | . | 4 | . | 3 | . | 112 |
| 782 | 36 | 29 | 5 | I | 4 | 8 | I | 3 | 14 | 13 | I | . | 3 | . | 2 | I | I | . | 3 | 2 | 127 |
| 783 | 64 | 62 | 2 | 8 | 7 | 5 | 13 | 14 | 15 | 26 | . | I | . | 4 | . | 4 | . | I | . | I | 240 |
| 784 | 58 | 64 | 6 | 6 | 7 | 2 | 6 | 4 | 10 | 11 | . | . | . | I | I | I | . | I | 2 | 2 | 184 |
| 785 | 29 | 32 | . | 5 | 2 | 2 | I | . | 8 | 10 | . | . | . | . | I | I | 2 | . | . | . | 93 |
| 786 | 44 | 36 | 3 | 3 | 7 | 9 | 4 | 2 | 7 | 9 | I | . | . | I | . | . | 2 | . | I | . | 130 |
| 788 | 40 | 46 | 6 | 5 | 5 | 9 | 8 | 7 | 9 | 11 | 2 | 2 | I | 2 | 4 | 3 | . | 2 | 4 | 2 | 168 |
| 789 | 52 | 39 | 5 | 4 | 4 | 6 | 8 | 7 | 11 | 14 | 2 | I | . | 3 | 3 | 3 | . | I | 2 | 2 | 170 |
| 790 | 107 | 85 | 2 | 5 | 6 | 7 | 5 | 9 | 20 | 22 | I | . | I | I | I | 4 | . | . | . | I | 290 |
| 791 | 52 | 51 | 8 | 6 | 5 | 5 | I | 5 | 14 | 14 | I | . | I | . | I | 6 | . | . | . | I | 164 |
| 793 | 28 | 29 | 5 | I | 3 | 4 | 5 | 6 | 9 | 21 | . | . | . | . | I | I | . | . | I | . | 115 |
| 795 | 30 | 49 | 4 | 9 | 8 | 13 | 15 | 6 | 13 | 14 | I | . | 2 | 2 | 5 | I | . | I | . | 2 | 187 |
| 797 | 26 | 28 | 3 | 3 | 8 | 5 | 6 | 6 | 6 | 11 | I | I | . | 2 | 2 | . | . | I | . | I | 113 |
| 799 | 16 | 10 | I | 3 | 2 | I | I | I | 2 | I | . | I | . | . | . | . | . | . | . | . | 39 |
| 800 | 43 | 59 | 3 | 2 | 2 | 7 | 3 | 9 | 9 | 15 | . | . | I | I | I | 2 | . | . | I | . | 159 |
| 901 | 34 | 43 | 3 | 5 | 6 | I | 4 | 4 | 14 | 12 | . | . | I | . | . | 4 | . | . | 2 | I | 135 |
| 902 | 47 | 57 | 3 | 6 | 2 | 3 | 8 | 7 | 20 | 15 | 3 | . | I | . | I | 2 | . | . | . | . | 175 |
| 903 | 13 | 10 | 2 | I | 3 | I | I | . | 4 | 4 | . | . | I | . | I | . | I | . | . | . | 42 |
| 904 | 23 | 31 | 3 | 2 | 3 | 2 | 2 | 4 | 12 | 4 | . | . | . | I | . | . | . | . | . | . | 87 |
| 905 | 65 | 55 | 7 | 4 | 5 | 7 | 8 | 5 | 22 | 21 | I | . | 2 | 3 | 4 | . | . | . | 2 | . | 212 |
| 906 | 45 | 34 | 3 | 5 | 9 | 5 | 16 | 4 | 14 | 11 | . | . | I | . | I | I | . | I | . | . | 164 |
| 907 | 52 | 50 | 2 | 9 | 8 | 13 | 7 | 8 | 15 | 17 | 2 | . | I | 2 | 3 | 2 | . | . | 2 | I | 207 |
| 908 | 57 | 50 | 6 | 7 | 5 | 7 | 3 | 4 | 7 | 15 | . | . | . | 2 | 5 | . | . | . | 2 | . | 170 |
| 909 | 37 | 48 | 4 | 7 | 5 | 4 | 9 | 11 | 18 | 15 | . | 2 | 6 | 2 | 3 | 5 | I | . | 5 | I | 195 |
| 910 | 45 | 44 | 7 | 4 | 6 | 6 | 5 | 5 | 9 | 11 | I | . | 2 | . | I | 2 | I | I | I | . | 151 |
| 911 | 35 | 34 | 3 | 2 | 3 | 6 | 3 | 5 | 11 | 7 | I | I | . | 2 | 3 | 2 | . | . | . | I | 119 |
| 912 | 27 | 31 | 4 | 5 | 2 | 2 | I | 3 | 3 | 6 | . | . | I | I | 2 | . | . | . | 3 | . | 92 |
| 913 | 19 | 19 | 5 | 4 | 2 | 6 | I | 3 | 10 | 10 | I | I | . | I | I | I | . | . | I | I | 88 |
| 914 | 13 | 19 | 2 | 2 | 3 | . | I | 2 | 12 | 8 | I | . | . | . | . | . | . | . | . | . | 63 |

TABLE D (continued)

| References | 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | 1,3,4 | 2,3,4 | 1,2,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 916 | 39 52 | 3 5 | 2 6 | 7 8 | 9 13 | 1 . | 1 2 | 2 1 | . 1 | 1 1 | . . | . . | . . | . . | . . | . . | 154 |
| 917 | 41 29 | 5 3 | 5 5 | 12 10 | 6 11 | 1 . | . . | 1 1 | 2 . | 1 . | 1 . | 1 . | . . | . . | . . | . . | 135 |
| 918 | 15 28 | 4 1 | 2 1 | 2 1 | 5 6 | . 1 | . . | . 1 | . . | . 1 | . . | . . | . . | . . | . . | . . | 68 |
| 920 | 21 22 | 4 3 | 1 5 | 3 . | 3 6 | 1 . | . . | . 1 | . . | . 1 | . . | . . | . . | . . | . . | . . | 71 |
| 921 | 21 17 | 2 4 | 6 3 | 3 5 | 3 2 | 1 1 | 1 1 | 2 2 | . 1 | 1 1 | 1 . | . . | . . | . . | . . | . . | 78 |
| 922 | 58 61 | 8 5 | 7 8 | 13 10 | 26 13 | . . | 2 1 | 4 3 | . . | 2 2 | . . | . . | . . | . . | . . | . . | 223 |
| 923 | 15 6 | 1 . | 1 1 | 2 1 | 2 1 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 31 |
| 924 | 28 51 | 4 6 | 10 9 | 7 6 | 9 14 | . 1 | 2 . | . 2 | . 1 | 1 . | . . | . . | . . | . . | . . | . . | 151 |
| 925 | 36 33 | 1 8 | 6 7 | 2 7 | 18 9 | 2 1 | . . | . 4 | . . | 2 . | . . | . . | . . | . . | . . | . . | 136 |
| 926 | 36 33 | 6 2 | 7 4 | 5 9 | 5 15 | . . | 1 1 | 2 1 | . . | 2 1 | . 1 | . . | . . | . . | . . | . . | 131 |
| 927 | 18 7 | 2 3 | 1 1 | 3 1 | 4 2 | . 1 | 1 . | . 1 | . . | . 1 | . . | . . | . . | . . | 1 . | . . | 47 |
| 929 | 63 39 | 1 2 | 3 3 | 3 4 | 10 10 | . . | . 1 | . 4 | 1 1 | 1 . | . . | . . | . . | . . | . . | . . | 146 |
| 930 | 39 35 | 6 6 | . 2 | 8 6 | 17 10 | . 1 | 1 1 | 3 3 | 1 . | 2 1 | . 2 | . . | . . | . . | . . | . . | 144 |
| 931 | 26 32 | 2 4 | 4 8 | 2 4 | 4 15 | 1 1 | . 1 | . 1 | . . | 1 . | . 1 | . . | . . | . . | . . | . . | 107 |
| 932 | 16 17 | . . | 2 3 | 2 2 | 8 5 | . . | . . | . 1 | . 1 | . . | . . | . . | . . | . . | . . | . . | 58 |
| 933 | 21 36 | 2 . | 6 2 | 2 5 | 5 6 | . . | 2 . | 3 1 | . . | . . | . . | . . | . . | . . | . . | . . | 91 |
| 934 | 15 18 | 2 4 | . 1 | 2 7 | 4 8 | . . | . 1 | 1 2 | . . | 1 . | . . | . . | . . | . . | . . | . . | 67 |
| 935 | 15 18 | 2 4 | . 1 | 2 7 | 4 8 | . . | . 1 | 1 2 | . . | 1 . | . . | . . | . . | 1 . | . . | . . | 67 |
| 936 | 22 23 | 4 6 | 5 2 | . 4 | 6 1 | . 1 | 1 . | . . | . . | 1 . | . . | 1 . | . . | . . | . . | . . | 77 |
| 940 | 30 20 | 4 2 | . 2 | 6 1 | 7 6 | . . | . . | 1 . | . . | 1 . | . . | . . | . . | . . | . . | . . | 80 |
| 941 | 19 29 | 3 6 | 3 5 | 6 1 | 2 6 | 1 . | 1 . | . 1 | 1 . | 1 1 | . . | . . | . . | . . | . . | . . | 86 |
| 942 | 21 23 | 3 4 | 6 7 | 5 9 | 11 9 | 1 1 | 2 1 | 2 2 | 2 . | . 2 | . 1 | . . | . . | . . | . . | . . | 112 |
| 943 | 21 20 | 4 3 | 5 3 | 1 3 | 6 9 | 1 1 | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 77 |
| 944 | 9 19 | 3 1 | 2 1 | 2 4 | 10 8 | . . | . . | 2 2 | . . | 2 . | . . | . . | . . | . . | . . | . . | 65 |
| 946 | 8 6 | 3 2 | . 6 | . 2 | 7 3 | 1 . | . 2 | . . | 1 . | 1 . | . . | . . | . . | 1 . | . . | . . | 43 |
| 948 | 21 18 | 1 5 | 2 6 | 3 1 | 8 4 | . 1 | . 1 | 3 2 | 2 . | 2 2 | . 1 | . . | . . | . . | . . | . . | 83 |
| 949 | 46 15 | . . | 2 2 | 2 2 | 5 3 | 2 . | . 1 | 1 1 | . . | . 1 | . . | . . | . . | . . | . . | . . | 83 |
| 950 | 13 17 | 3 4 | . 1 | 4 1 | 7 1 | . . | 1 . | 1 . | . . | . . | . . | . . | . . | . . | . . | . . | 53 |
| 951 | 17 24 | 2 6 | 4 2 | 2 2 | 8 11 | . . | 2 . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 81 |
| 952 | 20 26 | 2 2 | 3 2 | 7 3 | 10 8 | . 1 | . . | 1 . | . . | 1 . | 1 . | . . | . . | . . | . . | . . | 87 |
| 953 | 23 20 | 2 4 | 4 2 | 3 6 | 4 5 | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | . . | . . | 74 |
| 954 | 31 11 | . 2 | 1 . | 4 . | 13 4 | . 2 | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 69 |
| 957 | 26 25 | 1 . | 4 1 | 5 2 | 9 5 | . . | . . | 2 1 | . . | 1 . | 1 . | . . | . . | . . | . . | . . | 83 |
| 958 | 20 19 | 3 1 | . 4 | 5 6 | 5 7 | 1 . | . . | 2 . | 1 . | . . | . . | . . | . . | . . | . . | . . | 74 |
| 959 | 26 22 | 2 3 | 1 3 | 4 2 | 11 2 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 77 |
| 960 | 22 23 | 1 7 | 1 1 | 5 3 | 6 12 | 1 1 | . . | 1 . | . . | 1 . | . . | . . | . . | . . | . . | . . | 85 |
| 961 | 25 8 | 2 2 | 2 3 | 4 2 | . 2 | 1 . | . . | . 1 | . . | 1 . | . . | . . | . . | . . | . . | . . | 53 |
| 962 | 32 20 | 4 . | . 1 | 3 2 | 10 3 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 85 |
| 963 | 15 24 | 2 1 | 1 5 | 4 1 | 5 7 | 1 . | . . | . 1 | . . | 1 . | . . | . . | . . | . . | . . | . . | 68 |
| 964 | 19 14 | 3 1 | . 2 | 1 4 | 10 8 | . . | . . | 1 . | . . | 1 . | . . | . . | . . | . . | . . | . . | 64 |
| 965 | 26 15 | 1 1 | . 2 | 4 4 | 7 7 | . . | 1 . | . 1 | 1 . | . 1 | . . | . . | . . | . . | . . | . . | 71 |
| 966 | 23 21 | 4 4 | 4 . | 3 2 | 7 6 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 75 |
| 968 | 16 28 | 5 3 | 2 2 | 2 3 | 5 6 | . 1 | 1 . | . 1 | . . | 1 1 | . . | . . | . . | . . | . . | . . | 77 |
| 970 | 16 23 | 2 3 | 1 4 | 4 2 | 6 6 | 1 . | . . | . . | . . | 1 1 | . . | . . | 1 . | . . | . . | . . | 71 |
| 971 | 20 24 | 2 2 | 2 2 | 3 2 | 11 11 | . 1 | 1 . | . . | . . | . 3 | 1 . | . . | . . | . . | . . | . . | 85 |
| 973 | 23 30 | 3 3 | 1 2 | 3 1 | 6 6 | 1 . | 2 1 | 1 . | 1 . | . 2 | . . | . . | . . | . . | . . | . . | 86 |
| 974 | 16 19 | 2 2 | 3 5 | 2 2 | 4 4 | 1 . | . 1 | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 62 |
| 975 | 35 36 | 4 1 | 5 8 | 5 9 | 8 9 | 1 . | . . | . 1 | 1 1 | 1 . | . . | . . | 1 . | . . | . . | . . | 126 |
| 976 | 41 37 | 3 1 | 3 6 | 6 6 | 11 12 | 1 . | . . | 1 1 | . 2 | 1 2 | . . | . . | . . | . . | . . | . . | 134 |
| 982 | 41 22 | 4 2 | 2 4 | 4 3 | 7 4 | . . | 1 . | 2 1 | . . | . . | . 1 | . . | . . | . . | . . | . . | 98 |
| 983 | 23 22 | 3 5 | 7 4 | 3 5 | 13 5 | 1 . | . . | 1 3 | 1 . | . 3 | . . | . . | . . | . . | . . | . . | 99 |
| 984 | 22 30 | 5 3 | 4 3 | 4 11 | 7 6 | . . | . . | 1 2 | 1 . | . . | . . | . . | . . | . . | . . | . . | 99 |
| 985 | 29 24 | 5 5 | 3 3 | 6 3 | 11 5 | 1 . | 1 . | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | 97 |
| 986 | 24 28 | 6 2 | 7 5 | 5 3 | 13 10 | 1 . | . . | 1 . | . . | . 1 | . 1 | . . | . . | . . | . . | . . | 107 |
| 987 | 27 16 | 6 4 | 1 5 | 1 2 | 7 3 | 1 1 | 2 2 | 1 . | . . | 3 . | . . | . . | . . | . . | . . | . . | 82 |
| 989 | 16 14 | 1 1 | 6 . | 2 5 | 3 5 | . . | 1 . | 1 2 | . . | 1 . | . . | . . | . . | . . | . . | . . | 58 |
| 991 | 20 18 | 4 1 | 1 2 | 2 . | 3 7 | . . | . . | 1 . | . . | . 3 | . . | . . | . . | . . | . . | . . | 62 |
| 994 | 15 13 | 2 . | 2 2 | 4 4 | 1 4 | . . | . . | . . | . . | 1 . | 1 . | . . | . . | . . | . . | . . | 49 |
| 995 | 21 27 | . 3 | 2 5 | 1 1 | 9 9 | . . | 1 . | . 1 | . . | 1 1 | . 1 | . . | . . | . . | . . | . . | 83 |

TABLE D (continued)

| References | 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | 1,3,4 | 2,3,4 | 1,2,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 996 | 21 24 | 3 4 | 4 4 | 6 . | 10 11 | 1 . | . . | . 1 | 1 . | 1 1 | . . | . . | 1 . | . . | . . | . . | 93 |
| 997 | 29 28 | 2 1 | 8 6 | 3 3 | 7 3 | 2 . | . . | . 2 | 1 . | 1 1 | . 1 | . . | . . | . . | . . | . . | 98 |
| 998 | 26 26 | 3 . | 2 1 | 1 8 | 14 9 | . . | . . | 2 . | . . | 1 . | . 1 | . . | . . | . . | . . | . . | 94 |
| 999 | 24 27 | 4 7 | 2 5 | 1 4 | 6 5 | . 1 | 1 . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 87 |
| 1002 | 17 11 | 2 5 | 2 1 | 1 4 | 6 6 | . . | . . | 2 . | 1 . | . 1 | . . | . . | . . | . . | . . | . . | 59 |
| 1003 | 29 13 | 2 2 | 1 3 | 4 5 | 5 5 | 1 . | 2 . | 1 6 | . . | . 1 | . . | . . | . . | . . | . . | . . | 80 |
| 1005 | 26 20 | 2 3 | 4 2 | 3 4 | 8 6 | . . | . . | 1 . | . . | . 3 | . 1 | . . | . . | . . | . . | . . | 83 |
| 1010 | 10 16 | 3 1 | 3 4 | 7 . | 1 6 | . . | . . | . 1 | . . | 2 . | . . | . . | . . | . . | . . | . . | 54 |
| 1011 | 13 17 | 3 4 | 2 1 | 3 2 | 8 3 | . . | 2 . | 1 . | . . | 2 . | . . | . . | . . | . . | . . | . . | 61 |
| 1014 | 26 19 | 1 . | 1 4 | 2 1 | 7 7 | . . | 1 . | 1 . | . . | . . | . 1 | . . | . . | . . | . . | . . | 70 |
| 1023 | 14 22 | 4 3 | 2 4 | 3 . | 5 5 | 2 . | . . | 2 . | . . | . . | . 1 | . . | . . | . . | . . | . . | 67 |
| 1025 | 18 20 | 1 4 | 2 6 | 2 4 | 11 7 | 1 1 | . . | 1 1 | . 1 | 1 . | . . | . . | 1 . | . . | . . | . . | 82 |
| 1026 | 53 51 | 5 4 | 7 5 | 8 5 | 12 13 | . 1 | 1 . | 2 2 | . 1 | 1 1 | . . | . . | . . | . . | . . | . . | 172 |
| 1027 | 47 41 | 5 2 | 7 4 | 5 8 | 8 7 | 1 . | 1 . | 1 . | . . | 1 . | . . | . . | . . | . . | 1 . | . . | 139 |
| 1028 | 28 51 | 9 5 | 6 9 | 7 9 | 18 10 | 4 2 | 1 1 | 2 2 | . 1 | . 1 | . 1 | . . | . . | . . | . . | . . | 167 |
| 1029 | 40 35 | 5 6 | 5 2 | 9 10 | 11 9 | 2 . | 1 . | 2 2 | 1 . | 1 1 | 2 1 | . . | 1 . | . . | . . | . . | 146 |
| 1031 | 33 32 | 9 7 | 13 12 | 8 11 | 17 11 | 1 1 | 3 3 | 2 . | . . | 4 2 | 1 1 | . . | 1 . | . . | . . | . . | 173 |
| 1032 | 33 45 | 10 9 | 13 11 | 8 5 | 14 16 | 1 1 | . 3 | 3 1 | . . | 3 2 | 1 1 | . . | . . | . . | . . | . . | 180 |
| 1033 | 56 60 | 4 4 | 3 2 | 9 11 | 22 17 | . . | 1 1 | 6 3 | 1 . | . 1 | . . | . . | . . | . . | . . | . . | 201 |
| 1034 | 35 49 | 9 11 | 4 1 | 4 10 | 14 16 | 1 . | 3 1 | . . | 1 . | 1 1 | . 1 | . . | . . | . . | . . | . . | 162 |
| 1035 | 33 23 | 2 2 | 2 5 | 6 7 | 10 6 | 2 . | . 1 | 1 3 | . . | 1 . | . . | . . | . . | . . | . . | . . | 104 |
| 1036 | 58 52 | 4 4 | 9 8 | 6 8 | 7 13 | 2 1 | 1 2 | 2 3 | 2 . | 2 . | 1 . | . . | . . | . . | . . | . . | 185 |
| 1038 | 43 37 | 5 6 | 2 2 | 8 12 | 12 13 | . . | 4 . | . . | . 2 | . 1 | . . | . . | . . | 1 . | 1 . | . . | 148 |
| 1040 | 37 37 | 4 5 | 2 6 | 7 12 | 7 16 | . . | . 1 | . 3 | 1 1 | . 1 | . . | . . | . . | . . | . . | . . | 140 |
| 1041 | 46 41 | 9 4 | 4 2 | 8 10 | 9 14 | . 2 | . . | . 1 | . . | . 1 | . . | . . | 1 . | . . | . . | . . | 152 |
| 1042 | 35 48 | 5 2 | 6 4 | 6 4 | 1 20 | . 1 | . . | . . | . . | 3 1 | 1 . | . . | . . | . . | . . | . . | 137 |
| 1043 | 18 15 | 1 1 | 2 2 | 1 3 | 4 4 | 1 1 | . . | . 1 | . . | 1 . | . . | . . | . . | . . | . . | . . | 55 |
| 1046 | 28 35 | 6 4 | 2 7 | 10 6 | 4 3 | . . | . 2 | 3 2 | 1 . | . 1 | . . | . . | . . | . . | . . | . . | 114 |
| 1060 | 13 14 | 4 2 | 5 3 | . 2 | 4 4 | . 1 | . 1 | 1 1 | . 3 | 2 1 | 1 . | . . | 1 . | . . | . . | . . | 63 |
| 1063 | 25 43 | 6 4 | 4 12 | 4 8 | 2 6 | 1 2 | 4 2 | 1 3 | . . | . 1 | . . | . . | . . | . . | . . | . . | 128 |
| 1066 | 42 23 | 4 3 | 1 4 | 4 1 | 12 8 | . . | . 1 | 1 . | 1 . | 1 1 | . . | . . | . . | . . | . . | . . | 107 |
| 1071 | 46 50 | 5 4 | 3 4 | 5 7 | 17 21 | 1 . | . 3 | 1 . | . . | 3 3 | . 1 | . . | . . | . . | . . | . . | 174 |
| 1072 | 42 49 | 7 7 | 5 6 | 9 9 | 15 13 | . . | 1 . | 3 1 | . . | 1 3 | . . | . . | . . | . . | . . | . . | 171 |
| 1075 | 63 56 | 13 8 | 7 5 | 5 8 | 17 23 | . 5 | 5 2 | 1 2 | 1 1 | 1 1 | 2 1 | . . | 1 . | . . | . . | . . | 228 |
| 1084 | 50 21 | 1 4 | 3 3 | 1 1 | 8 6 | . 1 | . . | 3 . | . . | . . | . . | . . | . . | . . | . . | . . | 102 |
| 1088 | 68 50 | 7 7 | 7 3 | 10 9 | 15 21 | 2 . | 1 1 | . 3 | . 1 | . . | . . | . . | . . | . . | . . | . . | 214 |
| 1090 | 54 36 | 2 4 | 5 1 | 1 4 | 17 9 | 2 . | . . | . 2 | 3 . | . . | . . | . . | . . | . . | . . | . . | 140 |
| 1093 | 48 35 | 4 3 | 5 8 | 7 6 | 15 9 | 1 1 | . 1 | . 2 | . . | 1 1 | . . | . . | . . | . . | . . | . . | 147 |
| 1094 | 38 43 | 10 5 | 6 11 | 5 9 | 9 8 | 1 . | 2 2 | 3 1 | . 1 | 1 2 | . 1 | . . | . . | 1 . | . . | . . | 159 |
| 1095 | 56 58 | 4 8 | 8 5 | 7 5 | 11 8 | 5 . | 1 1 | 2 2 | . . | 4 1 | . . | . . | . . | . . | . . | . . | 184 |
| 1097 | 40 23 | 5 4 | . 4 | 5 7 | 8 14 | . 1 | 1 1 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | 114 |
| 1101 | 82 61 | 12 7 | 9 10 | 11 18 | 29 15 | 2 1 | 4 . | 5 2 | . . | . 2 | . . | 1 . | 1 . | . . | . . | . . | 272 |
| 1102 | 46 58 | 5 8 | 3 3 | 13 7 | 13 9 | . . | 1 2 | 2 1 | 1 . | . 3 | . . | . . | . . | . . | . . | . . | 175 |
| 1103 | 66 67 | 13 8 | 4 12 | 11 13 | 31 18 | . 1 | . 1 | . 3 | . . | . 1 | . . | . . | . . | . . | . . | . . | 249 |
| 1108 | 48 47 | 3 1 | 1 3 | 1 9 | 7 19 | 1 1 | . . | 2 . | . . | 1 . | 4 . | . . | . . | . . | . . | . . | 148 |
| 1109 | 52 51 | 7 5 | 8 9 | 8 10 | 23 19 | 2 1 | 3 1 | 3 . | 1 . | 1 1 | 1 1 | . . | . . | 1 . | . . | . . | 208 |
| 1112 | 43 31 | 5 2 | 6 4 | 7 4 | 6 5 | . . | 2 . | 1 1 | . . | . 1 | 1 . | . . | . . | . . | . . | . . | 119 |
| 1113 | 80 49 | 3 1 | 3 5 | 7 10 | 15 15 | . . | . 1 | 3 5 | 1 . | . 1 | . . | . . | . . | . . | . . | . . | 198 |
| 1116 | 48 47 | 2 2 | 5 6 | 7 11 | 19 15 | . . | 1 1 | 1 1 | . 1 | 1 1 | . 1 | . . | . . | . . | . . | . . | 170 |
| 1118 | 46 46 | 7 6 | 9 7 | 9 9 | 27 28 | 1 . | 3 2 | 3 2 | 1 . | 1 2 | . 1 | . . | . . | . . | . . | . . | 210 |
| 1119 | 28 36 | 3 8 | 4 3 | 3 6 | 7 8 | 1 . | . 1 | 2 2 | . 1 | . . | . . | . . | . . | . . | . . | . . | 113 |
| 1120 | 51 25 | 8 5 | 9 10 | 5 10 | 12 8 | 1 3 | 1 . | . 2 | . 1 | . . | . . | . . | . . | . . | . . | . . | 151 |
| 1121 | 68 70 | 6 9 | 4 13 | 10 6 | 18 14 | 1 1 | 2 2 | 2 . | 1 . | 1 . | . . | . . | . . | 1 . | . . | . . | 229 |
| 1131 | 86 95 | 4 17 | 7 11 | 13 15 | 32 34 | 2 . | 1 . | 2 1 | . . | 2 4 | 2 . | . . | . . | . . | . . | . . | 328 |
| 1133 | 81 co | 17 12 | 11 10 | 16 13 | 31 31 | 3 . | 3 3 | 3 3 | . . | 5 4 | . . | . . | . . | . . | 1 . | . . | 337 |
| 1139 | 57 63 | 9 7 | 2 1 | 8 14 | 18 9 | . . | . . | 2 . | . . | . 2 | . . | . . | 1 . | . . | . . | . . | 193 |
| 1141 | 31 26 | 6 5 | . 4 | 6 4 | 8 10 | . . | 1 1 | 2 . | . 1 | 2 . | . . | . . | . . | . . | . . | . . | 107 |
| 1142 | 21 46 | 8 6 | 3 6 | 5 8 | 11 14 | . 3 | 1 1 | 1 1 | 1 2 | 2 1 | . . | . . | . . | 1 . | 1 . | . . | 143 |
| 1148 | 38 37 | 6 1 | 1 3 | 8 9 | 10 11 | . . | . . | 1 1 | . . | 1 . | 1 . | . . | . . | . . | . . | . . | 128 |

TABLE D (continued)

| References | 0 | 1 | 2 | 3 | 4 | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | 1,3,4 | 2,3,4 | 1,2,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1150 | 30 50 | 4 4 | 4 5 | 10 6 | 15 14 | 1 . | . . | . 2 | . . | . 3 | . . | . . | . . | 2 . | . . | . . | 150 |
| 1151 | 28 30 | 2 1 | 3 4 | 7 6 | 9 4 | . 1 | 1 . | 1 . | . 1 | 1 . | . . | . . | . . | . . | . . | . . | 99 |
| 1152 | 71 60 | 6 7 | 6 8 | 17 10 | 14 18 | 1 1 | 2 3 | 2 3 | . . | . 1 | . 1 | . . | . . | . . | . . | . . | 231 |
| 1153 | 63 86 | 11 10 | 7 10 | 12 15 | 22 15 | 1 1 | . 2 | 2 2 | 1 . | 3 1 | . . | . . | . . | . . | . . | . . | 264 |
| 1154 | 44 45 | 6 10 | 4 3 | 8 12 | 15 14 | . 2 | 2 2 | 4 1 | . . | . . | . 2 | . . | 1 . | . . | 1 . | . . | 176 |
| 1156 | 58 52 | 8 8 | 8 5 | 9 7 | 15 22 | . . | . 1 | 1 1 | . . | 1 1 | 1 . | . . | . . | 1 . | . . | . . | 199 |
| 1158 | 33 18 | 2 3 | 5 5 | . 2 | 9 7 | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | . . | 85 |
| 1160 | 45 52 | 14 5 | 1 2 | 5 10 | 15 14 | . . | 2 . | 1 3 | . . | 1 . | . . | . . | . . | . . | . . | . . | 170 |
| 1165 | 32 16 | 4 1 | 5 5 | 15 1 | 11 11 | 1 . | 1 . | 1 . | 1 1 | 2 2 | 1 . | . . | 1 . | . . | . . | . . | 112 |
| 1169 | 26 19 | 3 2 | 3 5 | 6 8 | 5 5 | 1 1 | . 1 | 3 1 | 1 . | 1 . | 1 2 | 1 . | . . | . . | . . | . . | 95 |
| 1173 | 63 53 | 10 11 | 1 7 | 4 12 | 20 20 | . 2 | . 3 | 3 4 | . . | . . | 1 . | . . | . . | . . | . . | . . | 214 |
| 1174 | 80 73 | 10 8 | 3 11 | 16 14 | 25 35 | 4 . | 3 5 | 4 4 | . 1 | 1 4 | . . | . . | 2 . | 1 . | . 1 | . . | 305 |
| 1177 | 18 16 | 3 3 | 1 3 | 2 1 | 7 2 | 1 . | 1 . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 59 |
| 1179 | 40 41 | 5 4 | 4 8 | 7 4 | 15 8 | . . | . . | 1 3 | 2 . | . 2 | . . | . . | . . | . . | . . | . . | 144 |
| 1181 | 107 98 | 15 9 | 11 17 | 12 20 | 18 32 | 3 3 | 3 4 | 6 4 | . . | 1 . | . 1 | . . | . . | . . | . . | . . | 364 |
| 1182 | 95 106 | 12 15 | 15 16 | 16 19 | 25 26 | 1 . | 5 4 | 2 4 | . . | 1 1 | . 1 | . . | 1 . | . . | . . | . . | 365 |
| 1187 | 51 51 | 4 8 | 3 8 | 5 6 | 14 9 | . . | . 2 | 3 3 | . 1 | . . | 1 . | . . | . . | . . | . . | . . | 169 |
| 1188 | 39 24 | 2 2 | 8 4 | 2 4 | 12 9 | 1 . | 1 1 | 1 5 | 1 . | 1 . | . . | . . | . . | . . | . . | . . | 117 |
| 1199 | 46 39 | 6 5 | 8 12 | 8 15 | 25 10 | 1 . | . . | 1 3 | . 1 | 1 . | 1 1 | . . | . . | . . | . . | . . | 192 |
| 1200 | 40 31 | 4 4 | 5 5 | 10 9 | 9 8 | 1 . | 1 1 | 2 1 | . 1 | 2 1 | . . | . . | 2 . | . . | . 1 | . . | 138 |
| 1201 | 34 25 | 4 6 | 6 3 | 2 3 | 9 15 | . 1 | . . | 1 2 | . . | 1 1 | . . | . . | . . | . . | . . | . . | 113 |
| 1204 | 27 14 | 1 . | 3 1 | 3 1 | 2 2 | . . | . . | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | 55 |
| 1206 | 21 16 | 5 4 | 3 2 | 4 2 | 3 12 | . . | . . | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | 73 |
| 1219 | 9 14 | 4 1 | 4 8 | 1 3 | 4 6 | 1 1 | 2 1 | . . | . . | 1 . | . 1 | . . | . . | . . | . . | . . | 61 |
| 1220 | 44 40 | 4 4 | 6 9 | 1 6 | 14 14 | 1 . | . . | 2 4 | . 1 | 1 1 | . . | . . | . . | . . | . . | . . | 152 |
| 1221 | 12 13 | . 2 | . 1 | . 2 | 4 3 | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 37 |
| 1222 | 31 18 | 4 1 | . 4 | 1 6 | 5 9 | 1 . | . 1 | . 1 | . 1 | . . | . . | . . | . . | . . | . . | . . | 83 |
| 1225 | 21 13 | 2 2 | 1 3 | 1 2 | 4 7 | . . | . 2 | . . | 1 . | . . | . . | . . | . . | . . | . . | . . | 59 |
| 1230 | 11 20 | 1 7 | 2 3 | 2 1 | 4 7 | 1 . | . . | 1 1 | . . | . . | . . | . . | . . | . . | . . | . . | 64 |
| 1231 | 46 33 | 6 4 | 6 2 | . 3 | 13 10 | . . | 2 . | 1 2 | . . | 1 . | . . | . . | . . | . . | . . | . . | 129 |
| 1232 | 11 13 | 1 3 | 3 3 | 1 3 | 8 4 | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 50 |
| 1233 | 27 40 | 4 3 | 4 4 | 3 6 | 14 11 | 1 1 | 1 1 | . 2 | . . | . . | . . | . . | . . | . . | . . | . . | 127 |
| 1235 | 12 16 | 3 6 | 2 5 | 4 1 | 3 4 | 1 . | 1 . | 2 1 | . . | 1 . | . . | . . | . . | . . | . . | . . | 62 |
| 1236 | 25 19 | 2 7 | . 3 | 1 3 | 11 4 | . . | . . | . 1 | . 1 | . . | . . | . . | . . | . . | . . | . . | 77 |
| 1237 | 10 7 | 2 4 | 5 2 | 4 1 | 6 7 | . . | 1 . | 1 1 | . . | 1 . | 1 . | . . | . . | 1 . | . . | . . | 54 |
| 1238 | 12 14 | 2 1 | . 2 | . 1 | 3 4 | . . | . . | . 1 | . . | . . | . . | 1 . | . . | . . | . . | . . | 41 |
| 1240 | 27 27 | 3 4 | 4 9 | . 5 | 4 10 | . . | 1 . | 1 2 | . 1 | 2 3 | . . | . . | . . | . . | . . | . . | 103 |
| 1241 | 26 27 | 4 . | 3 2 | 2 6 | 6 13 | . . | 1 1 | 2 1 | . . | . . | . . | . . | . . | . . | . . | . . | 94 |
| 1243 | 44 36 | 5 3 | 4 3 | 7 2 | 18 15 | 2 . | 2 3 | 2 . | . . | . . | . . | . . | . . | . . | . . | . . | 147 |
| 1244 | 12 10 | 2 3 | 1 2 | 2 5 | 5 6 | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | 48 |
| 1247 | 30 30 | 2 5 | 4 5 | 4 4 | 9 9 | . . | 1 . | . 4 | . . | 2 1 | . . | . . | . . | . . | . . | . . | 109 |
| 1253 | 38 30 | 4 5 | 6 4 | 4 7 | 8 11 | 4 2 | 1 1 | 1 2 | . . | . 3 | . 1 | . . | . . | . . | . . | . . | 132 |
| 1254 | 33 28 | 7 8 | 2 6 | 4 8 | 7 11 | . . | . . | . . | 1 1 | . 1 | 1 . | . . | . . | . . | . . | . . | 118 |
| 1256 | 33 29 | 8 3 | 7 5 | 3 4 | 6 11 | 1 . | . . | . . | . . | 1 . | . . | . . | . . | . . | . . | . . | 111 |
| 1257 | 27 37 | 8 5 | 5 5 | 6 4 | 7 14 | 1 . | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | 120 |
| 1258 | 39 26 | 6 4 | 2 2 | 4 3 | 13 15 | 1 . | 1 2 | 4 1 | . . | . . | . 2 | . . | . . | . . | . . | . . | 125 |
| 1259 | 22 15 | 6 4 | 1 5 | 2 . | 12 14 | . . | . . | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | 72 |
| 1260 | 26 34 | 3 6 | 3 1 | 9 5 | 9 10 | . . | 1 2 | 1 . | . 2 | . . | . . | . . | . . | . . | . . | . . | 112 |
| 1261 | 22 16 | 3 7 | 2 . | 2 6 | 6 8 | 1 . | . 1 | . 1 | . 1 | 1 . | . . | . . | . . | . . | . . | . . | 77 |
| 1262 | 30 38 | 2 2 | 2 8 | 6 7 | 3 13 | 1 1 | 3 . | 1 . | . . | 2 1 | 1 . | . . | . . | . . | . . | . . | 121 |
| 1263 | 40 46 | 4 3 | 5 7 | 9 6 | 11 8 | 2 . | 1 1 | 3 2 | . . | 2 . | . . | . . | . . | . . | . . | . . | 150 |
| 1264 | 14 12 | 2 2 | 1 4 | 2 5 | 14 2 | . . | . . | . 1 | . . | . . | . . | . . | . . | . . | . . | . . | 59 |
| 1265 | 12 8 | 2 2 | 1 9 | 2 2 | 2 4 | 1 . | . . | . 2 | . . | 1 . | 1 . | . . | . . | . . | . . | . . | 49 |
| 1266 | 17 11 | . . | 3 4 | 4 2 | 2 2 | 1 . | 2 . | . 1 | 1 . | 1 3 | . . | . . | . . | . . | . . | . . | 54 |
| 1267 | 23 20 | 5 2 | 6 7 | 4 6 | 9 8 | . 2 | 1 2 | 1 1 | . . | 1 . | . 2 | . . | . 1 | . . | . . | . . | 101 |
| 1268 | 22 27 | 5 1 | 4 4 | 1 3 | 10 8 | . 1 | 1 1 | 4 2 | . . | 1 1 | . . | . . | . . | . . | . . | . . | 96 |
| 1281 | 39 50 | 8 14 | 7 6 | 4 10 | 10 17 | . 1 | . 2 | 1 4 | . . | 1 . | . . | . . | . . | . . | . . | . . | 174 |
| 1282 | 63 42 | 11 10 | 7 11 | 8 14 | 16 9 | 1 . | . 3 | . . | . 1 | 3 . | . 1 | . . | . 1 | . . | . . | . . | 207 |
| 1283 | 17 20 | 12 7 | . 4 | 3 3 | 4 6 | . . | . . | 1 . | . . | . 1 | . . | . . | . . | . . | . . | . . | 72 |

TABLE D (continued)

| References | 0 | | 1 | | 2 | | 3 | | 4 | | 1, 2 | 1, 3 | 1, 4 | 2, 3 | 2, 4 | 3, 4 | 1,2,3 | 1,2,4 | 1,3,4 | 2,3,4 | 1,2,3,4 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1284 | 28 | 9 | 2 | 2 | 1 | 5 | 2 | . | 4 | 2 | 3 | 2 | 1 | 1 | . | 1 | . | 1 | . | . | . | 66 |
| 1287 | 29 | 25 | 7 | 3 | 4 | 4 | 8 | 5 | 4 | 10 | . | 2 | 2 | 2 | 1 | 2 | . | 4 | . | . | . | 113 |
| 1288 | 64 | 21 | 1 | 2 | 4 | 4 | 5 | 6 | 7 | 8 | 2 | 1 | 1 | . | 1 | . | . | 1 | 2 | . | . | 130 |
| 1289 | 30 | 29 | 5 | 6 | 5 | 8 | 7 | 8 | 6 | 13 | 2 | . | 2 | . | 1 | . | . | . | . | 1 | . | 123 |
| 1290 | 42 | 38 | 9 | 9 | 10 | 7 | 8 | 11 | 15 | 14 | 4 | 2 | 1 | 2 | 3 | 2 | 1 | 1 | 2 | 3 | 2 | . | . | 1 | 2 | . | . | . | . | 189 |
| 1291 | 45 | 48 | 6 | 10 | 15 | 13 | 2 | 7 | 7 | 6 | 1 | 2 | 3 | 3 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | . | . | . | . | . | . | . | . | 180 |
| 1292 | 33 | 23 | 8 | 4 | 7 | 6 | 6 | 3 | 12 | 3 | 2 | 4 | 3 | . | 1 | 2 | . | 1 | 5 | 3 | 2 | 1 | . | . | . | . | . | . | . | 129 |
| 1293 | 41 | 35 | 7 | 5 | 9 | 8 | 9 | 4 | 11 | 8 | 3 | 1 | 1 | 1 | 1 | . | . | 2 | . | 2 | . | 1 | . | 1 | . | . | . | . | . | 150 |
| 1294 | 39 | 41 | 4 | 4 | 10 | 13 | 12 | 6 | 15 | 15 | . | 1 | 3 | 3 | 3 | 1 | . | . | 2 | 2 | 2 | 3 | 1 | . | 1 | . | . | . | . | 181 |
| 1296 | 40 | 43 | 11 | 4 | 1 | 9 | 4 | 6 | 15 | 16 | 3 | 1 | 3 | 1 | 4 | . | . | . | . | 2 | . | 2 | . | . | 1 | . | . | . | . | 166 |
| 1297 | 28 | 19 | 3 | 4 | 4 | 4 | 7 | 3 | 9 | 11 | . | . | . | 1 | 1 | . | . | . | 2 | 1 | . | . | . | . | . | . | . | . | . | 97 |
| 1298 | 47 | 71 | 6 | 8 | 6 | 6 | 11 | 8 | 11 | 12 | 1 | . | . | 1 | 4 | 2 | . | . | 2 | 3 | . | . | . | . | . | . | . | . | . | 199 |
| 1301 | 35 | 29 | 5 | 7 | 1 | 6 | 5 | 4 | 9 | 18 | . | 2 | 1 | 1 | 1 | 3 | . | 1 | . | 2 | . | . | . | . | . | . | . | 1 | . | 131 |
| 1302 | 32 | 24 | 6 | 2 | 2 | 5 | 2 | 2 | 2 | 11 | . | . | 1 | . | . | 1 | . | . | 1 | . | . | . | . | . | . | . | . | . | . | 91 |
| 1304 | 37 | 30 | 6 | 2 | 4 | . | 5 | 6 | 8 | 9 | 2 | . | . | 1 | . | . | . | . | . | . | . | . | . | 1 | . | . | . | . | . | 111 |
| 1305 | 33 | 30 | 6 | 7 | 3 | 7 | 9 | 7 | 6 | 5 | . | 4 | 1 | 2 | 2 | 2 | 1 | 3 | . | 2 | . | 1 | . | . | . | . | . | . | . | 131 |
| 1306 | 14 | 14 | 2 | 3 | 1 | 3 | 3 | 3 | 2 | 1 | . | 1 | 2 | 1 | . | 1 | . | . | 1 | 1 | . | 1 | . | . | . | . | . | . | . | 54 |
| 1307 | 44 | 41 | 7 | 7 | 6 | 9 | 10 | 9 | 12 | 14 | 1 | 1 | 4 | 1 | 2 | 1 | 1 | . | 2 | 1 | . | 1 | . | . | 1 | . | . | . | . | 175 |
| 1308 | 38 | 34 | 9 | 6 | 6 | 9 | 4 | 6 | 12 | 15 | 1 | . | 2 | . | 1 | 1 | . | 1 | 1 | . | . | . | . | . | 1 | . | . | . | . | 147 |
| Total | 17,171 | | 2208 | | 2211 | | 2639 | | 5163 | | 292 | | 392 | | 629 | | 132 | | 413 | | 125 | | 11 | | 39 | | 17 | | 13 | | 1 | | 31,456 |

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# SEX DETERMINATION IN THE WHITE FLY

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## INTRODUCTION

It is reported by WILLIAMS (1917) that in the common "white fly," *Aleurodes vaporariorum*, parthenogenetic eggs yield females among the English representatives of the species, but males in the United States. This statement is based on experiments with virgin females in both countries. On the other hand, two females of the English strain which had mated produced in the one family 13 females and 11 males, in the other family 2 females and 2 males. From these results WILLIAMS concludes that fertilized eggs of this species produce males and females in equal numbers, and applies his conclusion alike to the English and American strains. He also proceeds to explain several other well known instances of peculiar sex ratios in a similar manner, particularly among the Thysanoptera.

We have been unable to discover any other reported case of a species in which unfertilized eggs produce males while fertilized eggs yield both sexes. In such a species the males must be of two kinds, one produced from parthenogenetic eggs, the other from fertilized eggs. A species in which these two kinds of males existed would be invaluable in breeding experiments, owing to the presumably different capacities of the males for transmitting hereditary traits. For this reason we would not willingly

reject WILLIAMS's explanation of sex determination in Aleurodes, if we were not forced to do so. One of us (SHULL 1917) conducted numerous experiments on *Anthothrips verbasci* in the hope of finding that this species was of the kind that Aleurodes is reported to be, only to be driven to conclude that that thrips is precisely like the honey bee as regards sex. The case of Aleurodes seemed, therefore, to require further investigation.

The conclusion that the fertilized eggs of Aleurodes yield both sexes in equal numbers rested on the erroneous assumption that all eggs laid by mated females must be fertilized. It is well known that the female bee may and does lay unfertilized eggs while her spermatheca is full of spermatozoa; and SHULL (1917) found the same to be true of Anthothrips. If it be assumed that Aleurodes is like the bee and Anthothrips in this regard, the sex ratios obtained by WILLIAMS are quite as well explained as by his assumption that fertilized eggs may be of either sex.

To decide between these two possible explanations one might resort (1) to cytological study of the males, which on the one view should be all of one kind, and on the other view of two kinds probably differing in their chromosome number; or (2) to breeding experiments in which the males should transmit the characters of one parent or two, according as they came from parthenogenetic or fertilized eggs; or (3) to a study of the sex ratios obtained among the offspring of virgin and mated females. Believing that, if the facts should prove to be as we suspected they would, the third method entailed the least labor, we have employed the method of sex ratios. Let us see what sex ratios are permitted by the alternative theories.

(A) If Aleurodes is like the honey bee, and males come only from unfertilized eggs while all fertilized eggs produce females, the sex ratio in the family of a mated female may be anything whatever. Her offspring may be all females if every egg is fertilized, or they may be all males if in mating no spermatozoa are transferred or if the spermatozoa are withheld. Between these extremes may be any proportion of the sexes.

(B) If, as WILLIAMS believes, parthenogenetic eggs yield males, and fertilized eggs produce both sexes in equal numbers, the number of females could never exceed fifty percent. The maximum number of females would appear when all eggs were fertilized. If any developed parthenogenetically, the number of males would be increased, and the proportion of females would fall below fifty percent.

The method of sex ratios can only decide between these theories in case (A) is correct, and if in some instance a significant majority of females is obtained. If (B) is correct that fact can not be proved by sex ratios; and any proportion of females not over fifty percent fits either theory equally well. As shown below, we believe we have established such female majorities in several instances.

## METHODS

The white flies[1] were reared on bean and potato plants, and a few others, under lantern globes covered with fine gauze. To prevent the plants from growing too large during the rather long life cycle of the insects, and likewise to prevent fungus from developing under the covers, watering was reduced to a minimum. Virgin females were obtained by collecting pupae and permitting the adults to emerge in confinement. In experiments in which fertilized eggs were desired, in some cases mating was observed, in other cases the females were simply confined with males and pairing may or may not have occurred. In collecting the adult offspring for ascertaining their sex, if only a few were present, a brush and a bottle sufficed and but few specimens were lost. When large numbers were to be collected advantage was taken of the positive phototropism of the flies. The plant bearing them was placed in a tight box lined with black paper on all sides except one, into which a large glass funnel was fitted with stem outward. This funnel was turned toward the window and a bottle placed over the open stem. The flies gradually collected in this bottle, in which they were subsequently etherized. The repeated use of this cage in collecting flies from different plants made errors possible, and in one or two instances we suspect the results are incorrect for this reason; but great care was exercised to remove all flies from the box, and on the whole contamination seems to have been prevented.

## SEX RATIO IN RANDOM COLLECTIONS

Random collections in several greenhouses were made up of males and females in the proportions shown in table 1. Although the females are in the majority, the majority is small; and if the females live longer than the males, as WILLIAMS (1917) suggests, the sexes might have been present in equal numbers at the time of hatching. The "wild" collections

[1] The species used in our experiments is tentatively identified from adults, by Dr. A. L. QUAINTANCE and Dr. A. C. BAKER, as probably *Trialeurodes* (*Aleurodes*) *vaporariorum*. Unfortunately no pupae, upon which the positive determination depends, were preserved from the experiments.

TABLE 1

*Proportions of the sexes of Trialeurodes vaporariorum in random collections.*

| Place of collection | Number of males | Number of females |
|---|---|---|
| University greenhouse | 340 | 491 |
| Botanical greenhouse | 310 | 375 |
| Commercial greenhouse | 694 | 747 |
| Total | 1344 | 1613 |

do not, therefore, aid in deciding between the two theories of sex determination outlined above.

OFFSPRING OF VIRGIN FEMALES

Experiments in which virgin females were used as parents were conducted in two ways. In the first experiments the parents were left on the plants until the oldest offspring became adult, so that the parents may have appeared in the collections of progeny. In the later experiments

TABLE 2

*Showing the sex ratio of the offspring of virgin females, in experiments in which the parents may be included with the offspring.*

| Number of experiment | Date of beginning experiment | Number of virgin parents | Offspring | | |
|---|---|---|---|---|---|
| | | | Male | Female | Not determined |
| V1 | February 20 | 7 | 138 | 4 | 4 |
| V2 | February 21 | 8 | 298 | 6 | 24 |
| Total | | | 436 | 10 | 28 |

the parents were removed before any of the offspring matured, so that later collections included only the progeny. The early experiments, two in number, are recorded in table 2. In neither experiment is the number of females included among the offspring greater than the number of female parents used, so that the females recorded need not be offspring at all. In further support of this view is the fact that the females obtained were among the first collections, and that no subsequent females were found.

The experiments with virgin females, in which the parents were removed before the first offspring became adult are recorded in table 3. Our experiments confirm the statement of WILLIAMS that *unfertilized eggs produce only males.*

TABLE 3

*Showing the number of males and females among the offspring of virgin females, in experiments in which the parents were removed before any of the offspring became adult.*

| Number of experiment | Date of beginning experiment | Number of virgin parents | Offspring | | |
|---|---|---|---|---|---|
| | | | Male | Female | Not determined |
| V3 | February 23 | 3 | 2 | 0 | 0 |
| V3a | March 1 | 3 | 51 | 0 | 4 |
| V10 | April 19 | 2 | 28 | 0 | 0 |
| V20 | April 29 | 2 | 64 | 0 | 0 |
| V27 | May 11 | 5 | 2 | 0 | 0 |
| Total | | | 147 | 0 | 4 |

## OFFSPRING OF FEMALES THAT MATE

### *Mating not observed*

In a series of experiments mating was permitted to take place by putting males and females under the same cover, but was not observed to occur. The results of these experiments are collected in table 4. The

TABLE 4

*The offspring of females which were kept in the presence of males, but which were not observed to mate.*

| Number of experiment | Date of beginning experiment | Number of parents | | Offspring | | |
|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | Not determined |
| M2 | March 7 | 35 | 20 | 8 | 21 | 0 |
| M3 | March 7 | 15 | 9 | 47 | 39 | 5 |
| M6 | March 9 | 37 | 22 | 0 | 2 | 1 |
| M8 | March 25 | 31 | 25 | 37 | 27 | 1 |
| M9 | March 27 | 15 | 13 | 63 | 33 | 11 |
| M10 | March 30 | 35 | 34 | 63 | 53 | 5 |
| M11 | March 29 | 9 | 9 | 55 | 9 | 4 |
| M14 | March 30 | 30 | 23 | 351 | 324 | 8 |
| M22 | April 13 | 8 | 3 | 19 | 49 | 0 |
| M25 | April 17 | 11 | 3 | 56 | 76 | 0 |
| M26 | April 18 | 5 | 3 | 48 | 126 | 0 |
| M27 | April 19 | 4 | 2 | 86 | 68 | 0 |
| M28 | April 19 | 4 | 2 | 54 | 67 | 0 |
| M36 | May 10 | 8 | 2 | 2 | 0 | 0 |
| Total | | | | 889 | 894 | 35 |

only one of these experiments which exhibits a decided majority of females, and is therefore significant in deciding between the two alterna-

tive theories of sex determination outlined in the introduction, is M26. How significant this experiment is is indicated below.

### *Mating observed*

In the experiments shown in table 5, pairs of flies in copulation were separated, and the females alone put under lantern globes. Mating was thus known to have occurred, but could not have occurred repeatedly during the course of the experiment, as may have happened in table 4. Two of these experiments yielded a great majority of females.

TABLE 5

*The offspring of females which were observed in copulation, but which were then isolated so that further mating was prevented.*

| Number of experiment | Date of beginning experiment | Number of parents | Offspring | | |
|---|---|---|---|---|---|
| | | | Male | Female | Not determined |
| V15 | April 20 | 1 | 11 | 1 | 1 |
| V21 | April 30 | 3 | 66 | 205 | 0 |
| V26 | May 9 | 3 | 9 | 50 | 9 |
| Total | | | 86 | 256 | 10 |

The production of a large majority of females in the experiments of table 5 is probably owing to the fact that the parents had certainly mated before the beginning of the experiment. It suggests that in the experiments of table 4 some of the females did not mate at first, or may even not have mated at all, and that the small proportion of females was due to the laying of unfertilized eggs by such virgin females, rather than to the withholding of spermatozoa by females that had mated.

### SIGNIFICANCE OF THE EXPERIMENTS WITH MATED FEMALES

Three of the experiments with females that were either known to have mated or were given every opportunity to mate resulted in so large a majority of females as to appear to be decisive with regard to the method of sex determination. These are M26, V21, and V26. The fact that there were only three such experiments, whereas many others intended to be like them yielded only an equality or even a minority of females, does not diminish their value as evidence. Even if only one experiment in a thousand showed a majority of females, and that majority was sufficiently large, that one experiment would decide between the alternative views described in the introduction. It is important, therefore, to ascertain how significant the female majorities in these three cases are.

It may be objected that even if the two sexes were regularly produced in equal numbers, a random sample could, purely by chance, be composed of three to five times as many females as males. It may be argued that, even if there are in the spermatheca of a female fly two kinds of spermatozoa, respectively male- and female-producing, in equal numbers, the eggs laid may, purely by chance, be fertilized mainly by spermatozoa of the female-producing type. These chances do exist. How great they are can be computed.

Let us examine the totals of the three experiments, M26, V21, and V26. Assume that the flies recorded in tables 4 and 5 were drawn at random from an infinitely numerous population composed of equal numbers of males and females. The chance (probability) that any given sex ratio would occur in a sample is expressed by the formula

$$\frac{n!}{(n-r)!r!}\, p^r\, q^{(n-r)}$$

in which $n$ is the total number of individuals in the sample, $r$ is the number of females in such sample, $p$ is the chance that any selected individual will be a female, $q$ the chance that any selected individual will be a male. Under the conditions named above, $p = q = \frac{1}{2}$, while $n$ and $r$ vary with the samples.

Employing this formula, it is found that the chance that the 48 males and 126 females in experiment M26 might have come from a population in which males and females were equally abundant is

$$\frac{174!}{48! \times 126!}\,(\tfrac{1}{2})^{126}\,(\tfrac{1}{2})^{48} = \frac{174!}{48! \times 126! \times 2^{174}}$$

With the slight error due to the use of logarithms this chance proves to be 1 in 1,097,307,692. In like manner the chance that the ratio in experiment V21 could be 66 males and 205 females, and still represent an infinite population in which the sexes were equally numerous, is 1 in 310,942,857,142,857,142. The corresponding probability for experiment V26 is 1 in 45,876,666.

These figures, of course, only express the chance that the one specific ratio should occur. If one were to compute the chance that the ratio might be at least as far from equality as is the ratio actually obtained, it would be necessary to combine with the chance of the one ratio obtained the chances of all other ratios still farther from equality. Thus, in experiment V21, to the probability of the ratio 66 to 205 should be added

the probabilities of the ratios 65 to 206, 64 to 207, 63 to 208 . . . 1 to 270, 0 to 271. Since all these latter chances are less than that of the ratio 66 to 205, they may be combined and their sum still be only 1 in some quadrillions.

Contrast with these figures the chance that, in the same experiment (V21), the ratio might have been as near equality as possible, that is, 135 to 136. That chance is 1 in 20.6. Out of a large number of samples, of 271 individuals each, all drawn as hypothecated from an infinite population in which males and females are equally numerous, more than half of them should deviate less than 6 from equality. The number falling beyond the extreme ratios of 100 to 171, and 171 to 100, would be negligible.

It should be pointed out that the assumption of an infinite population from which to select the given samples is less favorable to the conclusion we propose to draw from the experiments than any finite population

TABLE 6

*Daily records of the progeny obtained in experiments M26, V21, and V26.*

| Experiment | Date of collection | Number of males | Number of females | Percentage of males |
|---|---|---|---|---|
| M26 | May 19 | 3 | 18 | 14.3 |
| | May 30 | 11 | 32 | 25.6 |
| | | 18 | 18 | 50.0 |
| | | 7 | 11 | 38.9 |
| | | 1 | 3 | 25.0 |
| | May 31 | 0 | 4 | 0.0 |
| | | 1 | 11 | 8.3 |
| | June 4 | 3 | 14 | 17.6 |
| | June 13 | 4 | 15 | 21.1 |
| | | 48 | 126 | 27.6 |
| V21 | May 30 | 7 | 58 | 10.8 |
| | | 6 | 15 | 28.6 |
| | | 0 | 6 | 0.0 |
| | June 17 | 26 | 62 | 29.5 |
| | | 24 | 51 | 32.0 |
| | | 2 | 11 | 15.4 |
| | June 20 | 1 | 2 | 33.3 |
| | | 66 | 205 | 24.4 |
| V26 | June 17 | 1 | 15 | 6.2 |
| | June 29 | 2 | 32 | 5.9 |
| | July 4 | 6 | 3 | 66.7 |
| | | 9 | 50 | 15.3 |

would be. If samples were drawn at random from any finite population, in which the sexes were equally abundant, the probability of any given one-sided ratio, such as 66 to 205, would be less than that computed above.

The above computations are based solely upon the total sex ratio obtained. They take no account of the nature of the individual collections within the experiments. These, however are important, and are given in table 6. It is noteworthy that with two exceptions each collection contained a majority of females. The fact that they are nearly all of the same sign enhances the probability that they represent a population in which the females are more numerous than the males.

The practical uniformity of sign of the several collections could be given expression and its significance shown by treating all the samples in each experiment by statistical methods. In view of the small number of collections in each experiment, however, the value of such treatment seems doubtful; and inasmuch as the computations already made appear wholly conclusive, we have dispensed with further refinements.

Experiment V26 may appear from table 6 to be less satisfactory as proof that the true proportion of males is less than 50 percent than are the other two experiments. But when it is observed (1) that most of the apparent weakness of this experiment lies in its last collection in which the males are in the majority, (2) that the females which produced these offspring mated only before and not during the experiment, and (3) that their supply of stored spermatozoa may have been exhausted by the fertilization of the earlier eggs, this final majority of males may well be regarded as a confirmation of the theory of sex determination which the preceding female majorities seem to render necessary, namely, that fertilized eggs yield females, unfertilized eggs males.

## DISCUSSION AND SUMMARY

The experiments described above indicate with little doubt that unfertilized eggs of *Trialeurodes vaporariorum* produce males, which confirms the statement of WILLIAMS with regard to the American representatives of this species. They should dispel, however, the belief that the fertilized eggs produce both sexes in equal numbers. If that belief were well founded, it should never be possible to obtain a significant majority of females, for to the fifty percent of males developed from fertilized eggs must be added the males which hatch from parthenogenetic eggs. In three experiments a very large majority of females was obtained. This result favors the view that, like the eggs of the

honey bee, all fertilized eggs of the white fly produce females, all parthenogenetic eggs males, in accordance with which view the sex ratios may be anything whatever. The only alternative explanations of the majorities of females in the three experiments above referred to would be selective fertilization and differential mortality. Since there is no indication that either of these phenomena occurs, and since analogy with the honey bee is at least probable, we have adopted the view that all fertilized eggs yield females.

While our conclusion is what we suspected at the outset that it would be, we believe that our judgment has been in nowise distorted by our preconception. We are, in fact, very desirous of discovering just such an animal as WILLIAMS describes this Trialeurodes to be. It is to be hoped that any indications that any species produces males from unfertilized eggs, and both sexes from fertilized eggs, will be promptly and thoroughly investigated.

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# INHERITANCE OF SPOTTED ALEURONE COLOR IN HYBRIDS OF CHINESE MAIZE[1]

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The color in the aleurone cells of maize early attracted the attention of geneticists as affording ideal material for testing the segregation of characters in the second generation of hybrids.

The first cases that were investigated led to the idea of only one unit or factor being involved in the production of an aleurone color. With the progress of investigation, however, it became necessary to assume that two factors are involved. The occurrence of red and purple seeds on the same ear showed the need of recognizing a third factor, and the appearance of a pale color, or "dilute," necessitated the assumption of a fourth, modifying, factor.

A brief examination of the many varieties grown by the Indians of North and South America shows that there are still other aleurone colors not provided for in this scheme, such as clay (a light blue but very distinct from dilute) found in varieties grown by the Hopi Indians of Arizona; pink (a light red), isolated from the Chinese waxy type, and black (an exceedingly intense blue or purple) found in varieties grown by the Navajo Indians and brown found in a Bolivian variety. It is not absolutely certain as yet whether additional factors are concerned in the production of these variations, or whether we are dealing with one or more dilution factors similar to those found in guinea pigs (WRIGHT 1916).

Apart from the many shades of color there are several color patterns corresponding in a certain sense to those of animals. Not all of these patterns have been, as yet, thoroughly investigated, but they are so distinct in appearance that there can be little doubt that additional factors or heritable elements are involved in their expression.

While at least two color schemes found on maize seeds are undoubtedly

[1] Since this manuscript was prepared a paper by Professor R. A. EMERSON (1918) has appeared, in which a similar type of aleurone color has been analyzed and designated mottled. In general the conclusions reached are similar to those presented here, but there are some differences which it is believed will be of interest.

patterns, two others would be classed more properly perhaps as a color dilution. If this is true there are two types of color dilution in the aleurone layer. The first of these has color in each aleurone cell but of greatly reduced intensity. This type is found in both red and blue. Most of the seeds belonging to it are darker at the base, the color paling gradually toward the tip. This type may possibly correspond to that designated parti-colored by EAST and HAYS (1911).

The other type of color dilution found in maize is perhaps comparable to the blue roan color in horses and cattle, in that with respect to color, there are two kinds of cells, those with pigment and those without. Unlike the blue roans, where cells of a color do not occur in groups, the seeds of maize are flecked with spots of irregular size, scattered indiscriminately over the entire surface and seemingly showing no evidence of concentration either at the base or the tip.

Cells of a color occur in groups of varying sizes. In many cases the color, however, is intense, the seed appearing lighter in shade only when groups of colored cells alternating with groups of colorless cells are extremely small. Cases occur in which the two types of dilution are combined, the seeds being spotted with a faint color.

Chemically it is quite possible that the two types of color dilution found in the aleurone layer of maize seeds represent two conditions of a single enzyme which causes the development of anthocyanin through the oxidation of a chromogen, a suggestion already advanced by WRIGHT (1917) to account for a parallel case in the melanin pigment of mammals.

Where the intensity of the pigment itself is reduced, the entire seed being but faintly colored, it may be that the enzyme is weak and slow to react.

While in the spotted type of dilution in which the color is intense but does not extend over the entire seed, it would appear that the enzyme is sufficiently active, but the quantity may be reduced to such an extent that enough chromogen can not be oxidized to cover the entire seed.

The occurrence of spotted seeds in a cross between the Chinese white waxy corn and a colored Algerian pop corn has given the opportunity to study the inheritance of this spotted type of color distribution.

There are several peculiarities in the production of spotting which are not inharmonious with the supposition that this type of color distribution is in reality a color dilution, due perhaps to a reduced quantity of an enzyme concerned in the production of color.

The factors which influence the appearance and production of color

in the aleurone cells of maize, so far as investigated and reported, are listed below:

*C*, a factor for the production of any color, but inactive unless another factor (which has been designated *R*) is present.

*R*, a factor as necessary as *C* for the production of color. With *C* it will produce a red color. It may be distinguished from *C* because the latter factor is correlated with the texture of the endosperm.

*A*, a factor reported by Emerson (1918) as necessary for the production of any color.

$P_r$, a factor that in combination with the factors *C* and *R* changes the color of the aleurone from red to blue or purple.

*I*, a factor concerned with the inhibition of color. It is assumed to affect the production of both red and blue.

*S*. This factor is here reported for the first time. It is concerned with the production of a certain type of minute spotting and seems to be associated or correlated with the factor *R*.

In 1910 crosses were made between a variety of maize received from Algeria, having colored aleurone and horny endosperm and a Chinese variety with white waxy endosperm. One cross was made with the Algerian variety as the male parent, the other with this variety as the female parent. Both of the resulting ears were uniform or self-colored, in this respect resembling the Algerian parent. The progeny of the ear having the Algerian plant as the male parent failed to show the usual association of endosperm texture and aleurone color.

This result seemed to afford ground for the belief that there might be a difference between reciprocal crosses with respect to the association of aleurone color and endosperm texture. To investigate this possibility, six crosses were made between these same two varieties in 1913. While no differences in the association of the characters were found between reciprocal crosses, the results showed conclusively that in these hybrids aleurone color may show different distributions in the seeds of reciprocal crosses.

Of the six hybrid ears three were borne on plants of the Algerian variety, the other three on plants of the Chinese variety. The former were self-colored and could not be distinguished from pure Algerian ears. The latter were also colored, but the color on two of these was in numerous small spots. Of these two, one had all the seeds spotted, the other approximately half of the seeds spotted, the actual ratio being 107 spotted to 97 self-colored. The relationship of the six ears is shown in figure 1.

It is to be noted that the Algerian plants, which bore the self-colored

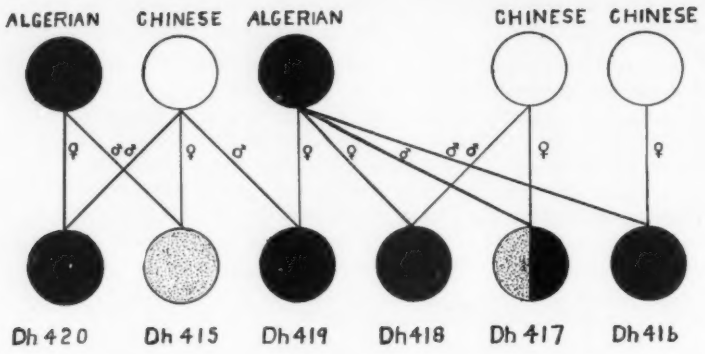


FIGURE 1.—Diagram showing the relationship of the 6 hybrid ears.

ears Dh420 and Dh419, showed an incomplete dominance when used as male parents with some Chinese plants, but a complete dominance when these same Chinese plants were used as male parents. That the dominance of the Algerian color is not always incomplete when Algerian plants serve as male parents is demonstrated by the hybrid ear Dh416 which had self-colored kernels.

Plants from all six ears were grown in 1914, and a large number of hand-pollinated ears bearing seeds representing the second generation were secured. The progeny of all but one of the six ears produced ears with some spotted seeds. The one exception, ear Dh416, was borne on a Chinese plant and was pollinated from the same Algerian plant that served as the male parent of ear Dh417, which was spotted.

The progeny of the remaining 5 ears produced 60 hand-pollinated ears having some of the seeds spotted. Of these 35 showed a dihybrid ratio of white to colored seeds, and 2 had all the seeds colored. It must be borne in mind, however, that these 60 ears were the result of self-pollination, and crosses between plants grown from the other hybrid ears.

As both red and blue seeds were found on ears having a dihybrid ratio of white to colored seeds, it was necessary to assume that three factors are involved in the production of color: (1) A basic factor ($C$), which is essential for the production of any color, (2) a factor for red ($R$) which in conjunction with the factor $C$ results in a red seed; and (3) a factor for blue ($P_r$), which, when present in the same zygote with the factors $C$ and $R$, results in these seeds being blue. All com-

binations of these factors other than *C* and *R*, or *C*, *R*, and $P_r$, result in the production of white seeds. The suggestion has been advanced that the factor correlated with endosperm texture is the basic factor *C* (KEMPTON 1915, BREGGER 1918). This is mentioned here, as it affords a method of distinguishing between the factors *C* and *R*, which is of

TABLE 1

*Percentage of spotted seeds on ears with a dihybrid ratio of white to colored seeds.*

| Pedigree number | Total number | Number colored | Number spotted | Percentages of total spotted | Percentage of colored that are spotted | Percentage of white |
|---|---|---|---|---|---|---|
| 2250 | 86 | 52 | 5 | 5.8 | 9.6 | 39.5 |
| 2261 | 648 | 369 | 104 | 16.1 | 28.2 | 43.0 |
| 2262 | 438 | 252 | 53 | 12.1 | 21.1 | 42.4 |
| 2263 | 251 | 153 | 36 | 14.3 | 23.5 | 39.0 |
| 2264 | 517 | 278 | 75 | 14.5 | 27.0 | 46.4 |
| 2265 | 345 | 199 | 49 | 14.2 | 24.6 | 42.3 |
| 2266 | 606 | 332 | 83 | 13.7 | 25.0 | 45.2 |
| 2301 | 218 | 124 | 35 | 16.1 | 28.2 | 43.2 |
| 2302 | 435 | 259 | 86 | 19.8 | 33.2 | 40.4 |
| 2311 | 178 | 110 | 28 | 15.7 | 25.4 | 38.2 |
| 2312 | 244 | 131 | 31 | 12.7 | 23.7 | 46.4 |
| 2315 | 508 | 288 | 100 | 19.7 | 34.7 | 43.3 |
| 2316 | 480 | 254 | 85 | 17.7 | 33.4 | 47.1 |
| 2324 | 283 | 168 | 61 | 21.5 | 36.3 | 40.6 |
| 2325 | 159 | 89 | 30 | 18.9 | 33.7 | 44.0 |
| 2326 | 309 | 177 | 55 | 17.8 | 31.1 | 42.7 |
| 2331 | 294 | 174 | 46 | 15.6 | 26.4 | 40.8 |
| 2332 | 526 | 286 | 104 | 19.8 | 36.4 | 45.6 |
| 2336 | 392 | 221 | 72 | 18.3 | 32.6 | 43.6 |
| 2339 | 595 | 358 | 129 | 21.7 | 36.1 | 39.8 |
| 2340 | 537 | 285 | 97 | 18.1 | 34.0 | 47.0 |
| 2342 | 357 | 208 | 70 | 19.6 | 33.6 | 41.7 |
| 2343 | 265 | 139 | 43 | 16.2 | 30.9 | 47.5 |
| 2347 | 395 | 226 | 80 | 20.3 | 35.4 | 42.8 |
| 2348 | 44 | 26 | 5 | 11.4 | 19.2 | 41.0 |
| 2350 | 311 | 165 | 64 | 20.6 | 38.8 | 47.0 |
| 2351 | 52 | 29 | 10 | 19.2 | 34.5 | 44.3 |
| 2352 | 565 | 320 | 89 | 15.8 | 27.8 | 43.4 |
| 2353 | 230 | 135 | 73 | 31.7 | 54.1 | 41.3 |
| 2356 | 454 | 268 | 80 | 17.6 | 29.8 | 41.0 |
| 2358 | 251 | 148 | 44 | 17.5 | 29.7 | 41.0 |
| 2359 | 512 | 309 | 105 | 20.5 | 34.0 | 39.6 |
| 2364 | 526 | 286 | 75 | 14.3 | 26.2 | 45.6 |
| 2365 | 34 | 13 | 3 | 8.8 | 23.1 | 61.7 |
| 2366 | 407 | 252 | 89 | 21.8 | 35.3 | 38.1 |
| Total | 12452 | 7083 | 2194 | 17.6 | 30.4 | 43.2 |

importance in analyzing the inheritance of spotted seeds, as will appear later.

All the ears with a dihybrid ratio of white to colored seeds had some seeds that were spotted. The percentage of the total seeds that showed spotting was 17.6 ± .236, but since spotting can be detected only when the seeds are colored, it would seem that the percentage of spotted seeds on an ear should be determined by the percentage of colored seeds that are spotted. If this assumption is admitted the percentage of spotted seeds becomes 30.4 ± .277 for these hybrid ears.

The ears having a monohybrid ratio of white to colored seeds fell into two groups as regards spotted seeds—those having some of the seeds spotted and those without any spotted seeds.

Disregarding for the present those ears without spotted seeds, the percentage of spotted seeds on the others is 21.7 ± .27, but the per-

TABLE 2

*Percentage of spotted seeds on ears with a monohybrid ratio of white to colored seeds.*

| Pedigree number | Total number | Number colored | Number spotted | Percentages of total spotted | Percentage of colored that are spotted | Percentage of white |
|---|---|---|---|---|---|---|
| 2251 | 268 | 204 | 63 | 23.5 | 30.8 | 23.9 |
| 2252 | 39 | 29 | 8 | 20.5 | 27.6 | 25.6 |
| 2256 | 627 | 484 | 92 | 14.7 | 19.0 | 22.8 |
| 2257 | 574 | 436 | 138 | 24.0 | 31.7 | 24.0 |
| 2259 | 576 | 421 | 103 | 17.9 | 24.5 | 26.9 |
| 2260 | 518 | 397 | 87 | 16.8 | 21.9 | 23.4 |
| 2303 | 267 | 185 | 44 | 16.5 | 23.8 | 30.7 |
| 2308 | 600 | 440 | 145 | 24.2 | 33.0 | 26.6 |
| 2319 | 402 | 279 | 111 | 27.6 | 39.8 | 30.6 |
| 2333 | 579 | 403 | 155 | 26.8 | 38.5 | 30.4 |
| 2334 | 260 | 193 | 70 | 26.9 | 36.2 | 25.8 |
| 2335 | 399 | 293 | 93 | 23.3 | 31.7 | 26.5 |
| 2337 | 497 | 386 | 132 | 26.6 | 34.2 | 22.3 |
| 2338 | 400 | 292 | 83 | 20.8 | 28.4 | 27.0 |
| 2344 | 564 | 409 | 129 | 22.9 | 31.5 | 27.5 |
| 2346 | 588 | 452 | 145 | 24.7 | 32.1 | 23.1 |
| 2349 | 605 | 432 | 112 | 18.5 | 25.9 | 28.6 |
| 2355 | 202 | 156 | 48 | 23.7 | 30.8 | 22.7 |
| 2357 | 288 | 221 | 49 | 17.0 | 22.2 | 23.3 |
| 2360 | 618 | 466 | 142 | 23.0 | 30.5 | 24.6 |
| 2362 | 554 | 408 | 129 | 23.3 | 31.6 | 26.4 |
| 2363 | 225 | 161 | 52 | 23.1 | 32.3 | 28.4 |
| 2345 | 471 | 313 | 66 | 14.0 | 21.1 | 33.5 |
| Total | 10121 | 7460 | 2196 | 21.7 | 29.4 | 26.3 |

centage of colored seeds that are spotted is 29.4 ± .30, essentially the same percentage as was observed on the ears with a dihybrid ratio of white to colored seeds (see table 2). The percentage of spotted seeds based on the total can not, of course, be alike on ears with monohybrid and dihybrid ratios of white to colored seeds, since the latter class has a relatively larger percentage of the total seeds white.

A plant of the hybrid Dh420 when crossed with a plant of the hybrid Dh417 resulted in the production of an all-colored ear. A similar result was secured when a plant of the hybrid Dh415 was crossed with another plant of the hybrid Dh417. The assumption in these cases is that one of the plants is genotypically constituted *CCRr* and the other *CcRR*. These two all-colored ears had 29.1 ± .82 of the seeds spotted (table 3). They are represented diagrammatically in figures 2 and 3 (pp. 272, 273).

Thus it is seen that ears with a dihybrid and those with a hybrid ratio of white to colored seeds, as well as ears having all of the seeds colored, have essentially the same percentage of the colored seeds spotted, provided, of course, that they have spotted seeds at all.

Combining the three classes of ears, those with a monohybrid and dihybrid ratio of white to colored seeds as well as the two ears having all the seeds colored, the percentage of colored seeds that are spotted is found to be 29.9 ± .19. This percentage at first glance might be considered an approximation of the 25 percent expected for a simple monohybrid ratio, but the deviation is 23.8 times the probable error.

TABLE 3

*Percentage of spotted seeds on ears having all the seeds colored.*

| Pedigree number | Total number | Number colored | Number spotted | Percentages of total spotted | Percentage of colored that are spotted | Percentage of white |
|---|---|---|---|---|---|---|
| 2254 | 699 | 699 | 191 | 27.3 | 27.3 | 0 |
| 2361 | 608 | 608 | 189 | 31.1 | 31.1 | 0 |
| Total | 1307 | 1307 | 380 | 29.1 | 29.1 | 0 |

As all of the ears having a dihybrid ratio of white to colored seeds had some of the seeds spotted and, further, as approximately one-half of the ears with a monohybrid ratio of white to colored seeds (the progeny of the hybrid Dh416 omitted) had some of the seeds spotted, it would appear that when a plant is heterozygous for one of the factors for aleurone color some of the seeds are spotted.

Confining the analysis to those ears which were the result of self-pollination it is found that when there is no correlation between aleurone color and endosperm texture all the ears have some spotted seeds, and conversely (if two ears having only slightly more than one percent of the seeds spotted are disregarded) when there is a correlation, none of the ears has spotted seeds. The distribution is then as follows:

| | Some seeds spotted | No seeds spotted |
|---|---|---|
| Aleurone color and endosperm texture correlated..... | 0 | 8 |
| Aleurone color and endosperm texture not correlated..... | 7 | 0 |

The factor for color correlated with endosperm texture has been designated *C*; therefore, when an ear is secured with a correlation between aleurone color and endosperm texture, the parents must have been heterozygous for the factor *C*, and if the ratio of colored to white seeds approximates 3 to 1, the parent must also have been homozygous for the factor *R*.

The above four-fold distribution establishes the fact that in the presence of a spotting factor, *S*, spotted seeds occur only when the factor uncorrelated with endosperm texture, *R*, is heterozygous. Since in these ears the factor *C* is homozygous, it remains to ascertain whether it is the homozygous *C* or the heterozygous *R* that determines the occurrence of spotting. This question is answered by the fact that spotted seeds are always found on ears with a dihybrid ratio of white to colored seeds. Since the parents of such ears were heterozygous for *C*, it follows that spotting is not dependent on *C* being heterozygous, and the alternative must be accepted, that spotting occurs only when the factor *R* is heterozygous.

A further refinement of this statement is necessary to account for spotted seeds on the two ears which were the result of crossing two plants, one genotypically constituted *CCRr*, the other *CcRR*. These ears were of course all colored but had 29.1 percent of the seeds spotted (table 2).

It is found that when the self-pollinated female parent produces some of the colored seeds spotted, and the male parent all the colored seeds self-colored, crossing these two will result in the production of an ear having some of the colored seeds spotted; but if this parentage is reversed

the female parent, when self-pollinated, producing all of the colored seeds self-colored, and the male parent producing some of the colored seeds spotted, a cross between them will have all the colored seeds self-colored. This harmonizes with the assumption that spotting can be detected only when the maternal parent is heterozygous for the factor *R* or homozygous for its allelomorph *r*. On this assumption the Chinese parent of the hybrids Dh417 and Dh418 must have been heterozygous for the factor *R*, and since both monohybrid and dihybrid ears were produced, all having correlations between aleurone color and endosperm texture, the genotypic composition must have been *ccRrss*, and the genotypic composition of the Algerian parent of these hybrids must have been *CCRRSS*.

The Chinese parent of Dh415 and Dh420 must have had the genotypic composition *Ccrrss*, the Algerian parent having the composition *CCRRSS*, since the progeny of these reciprocals had both monohybrid and dihybrid ratios, but the ears with a monohybrid ratio of white to colored had no correlation between aleurone color and endosperm texture. These ears were, therefore, homozygous for the factor *C*.

Since the hybrid Dh416 was not spotted and its progeny all had a monohybrid ratio of white to colored seeds, and since all of these ears had a correlation between aleurone color and endosperm texture, the Chinese parent must have been genotypically constituted *ccRRss*, and the Algerian parent must have been constituted *CCRRSS*, similar to the other Algerian plants. The progeny of this cross are all homozygous for the factor *R;* no spotted are to be expected and none was observed.

The evidence therefore appears complete that spotted seeds occur only when the female parent is heterozygous for the factor uncorrelated with endosperm texture, this factor being designated *R*.

It is obvious from the genotypic composition necessary for the appearance of this type of spotted seeds that a strain having all of the colored seeds spotted can not be obtained, although it is possible to secure strains that will always have some of the colored seeds spotted.

Thus, spotting can be observed when the female gamete is of the class *CrS*, and the male gamete *cRS, CRS, cRs*, or *CRs*, or the female gamete of the class *Crs* and the male gamete either *cRS* or *CRS*, or the female gamete *crS* and the male gamete *CRs* or the female gamete *crs* and the male gamete *CRS*. A plant which will produce these gametic classes must be heterozygous for the factor *R*, and since this is true some of the female gametes will be of the class *CRS*. And though the zygote will be colored regardless of the composition of the male gamete, it will not be spotted.

Since in this type of spotting the zygotes are heterozygous for the factor *R*, no ears bearing all the seeds colored should be obtained from self-pollinating plants grown from spotted seeds, while if the spotted seeds were not heterozygous for the factor *R*, self-pollinating plants grown from spotted seeds, secured from ears with a ratio of 3 colored to 1 white, should give one third of the ears with all the seeds colored. At the present time 90 plants have been grown from spotted seeds of these hybrids, but none of the ears from these plants has had all of the colored seeds spotted or all of the seeds colored.

As a further test several plants grown from spotted seeds were crossed with a strain called "*R* tester" secured from R. A. EMERSON and all proved to be heterozygous for *R*. It is known that varieties of maize having all of the seeds minutely spotted are grown by the North American Indians. These varieties breed true for spotting, and it is hoped that by the end of another season it will be possible to determine the relationship of this type of spotting to that represented by the hybrids under discussion.

### ASSOCIATION OF SPOTTING AND ALEURONE FACTORS

Having satisfied ourselves that spotting appears only when the female parent is heterozygous for the factor for aleurone color, *R*, attention may be turned to the rather unusual percentage of spotted seeds. The percentage of colored seeds that are spotted prevents any very simple explanation on the basis of multiple factors.

A satisfactory approximation of the observed percentage may be obtained by assuming a coupling or linkage between the factor for aleurone color, *R*, and a dominant spotting factor, *S*.

The best fit is secured by assuming the gametic ratio to be 7 : 1 : 1 : 7, the combination *RS* and *rs* being 7 times as numerous in the gametes as the combinations *rS* and *Rs*. This ratio may be the result of the two factors being located on the same chromosome and separated by 12.5 units, or the cells bearing the associated factors may divide or reduplicate 7 times as fast as the cells bearing the odd combinations.

On this assumption a plant which, when self-pollinated, produces an ear with a monohybrid ratio of white to colored seeds, with no correlation between aleurone color and endosperm texture, and having some spotted seeds, would be making the gametic classes shown in diagram 1.

Such a series will give 135 self-colored to 57 spotted seeds or 29.68 percent of the colored seeds spotted, which is a very close approximation to the observed percentage 29.4 ± .30.

| | 7 *CRS* | 1 *CRs* | 1 *CrS* | 7 *Crs* |
|---|---|---|---|---|
| 7 *CRS* | Self | Self | Self | Self |
| 1 *CRs* | Self | Self | Self | Self |
| 1 *CrS* | Spot | Spot | White | White |
| 7 *Crs* | Spot | Self | White | White |

DIAGRAM 1.

Continuing with the assumption that the factor for color, *R*, and the factor for spotting, *S*, are correlated in the gametes in a ratio of 7 : 1 : 1 : 7, self-pollinating a plant heterozygous for both color factors, *C* and *R*, as well as the spotting factor, *S*, would give an ear with the following zygotic classes (Diagram 2):

| | 7 *CRS* | 1 *CRs* | 1 *CrS* | 7 *Crs* | 7 *cRS* | 1 *cRs* | 1 *crS* | 7 *crs* |
|---|---|---|---|---|---|---|---|---|
| 7 *CRS* | 49 Self | 7 Self | 7 Self | 49 Self | 49 Self | 7 Self | 7 Self | 49 Self |
| 1 *CRs* | 7 Self | 1 Self | 1 Self | 7 Self | 7 Self | 1 Self | 1 Self | 7 Self |
| 1 *CrS* | 7 Spot | 1 Spot | 1 White | 7 White | 7 Spot | 1 Spot | 1 White | 7 White |
| 7 *Crs* | 49 Spot | 7 Self | 7 White | 49 White | 49 Spot | 7 Self | 7 White | 49 White |
| 7 *cRS* | 49 Self | 7 Self | 7 Self | 49 Self | 49 White | 7 White | 7 White | 49 White |
| 1 *cRs* | 7 Self | 1 Self | 1 Self | 7 Self | 7 White | 1 White | 1 White | 7 White |
| 1 *crS* | 7 Spot | 1 Spot | 1 White | 7 White | 7 White | 1 White | 1 White | 7 White |
| 7 *crs* | 49 Spot | 7 Self | 7 White | 49 White | 49 White | 7 White | 7 White | 49 White |

DIAGRAM 2.

The ratio of self-colored to spotted seeds is 405 to 171, the same ratio as in the monohybrid ears and in accord with the observed ratios. From this evidence it would not seem unreasonable to assume that the spotting

on these seeds is due to a dominant partial-inhibiting factor which is linked or coupled with the factor for color, *R*, in a ratio closely approximating 7 : 1 : 1 : 7.

For the two ears with all seeds colored obtained by crossing two first-generation plants, both of which when self-pollinated produce ears with a monohybrid ratio of white to colored seeds, the percentage of spotted seeds should be reduced from 29.68 to 28.1.

These two ears are represented in figures 2 and 3.

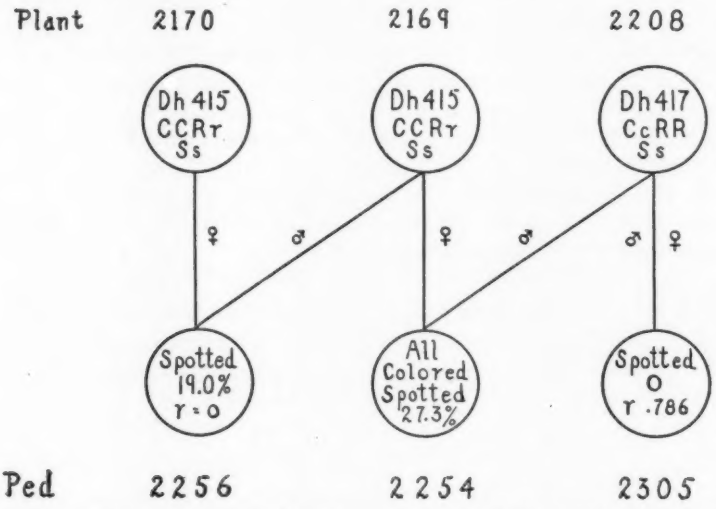


FIGURE 2.—Showing the relationship of ear 2254 which had all the seeds colored. The female parent of this ear when used as the male parent of 2256 produced 19.3 percent of the colored seeds spotted and produced no spotted seeds when used as the male parent of ear number 2305. The coefficients of association indicated on the ears 2256 and 2305 refer to the correlation between endosperm texture and aleurone color.

It is seen from this diagram that the male parent was making gametes *CRS, CRs, cRS, cRs*, and the female parent *CRS, CRs, CrS, Crs*. It is observed that no reduplication can occur in the gametes of the male parent between the factors *R* and *S*, as all of the gametes possess the factor *R*. In the female gametes, however, a 7 : 1 : 1 : 7 reduplication can be obtained. The gametic composition of the parents and the resulting zygotic classes are shown in diagram 3.

Were it not for the peculiar fact that spotting can appear only when the female gametes do not possess the factor *R*, the result of the gametic reduplication in the female gametes would not be apparent in the zygotes, and 37.5 percent of the seeds would be expected to be spotted.

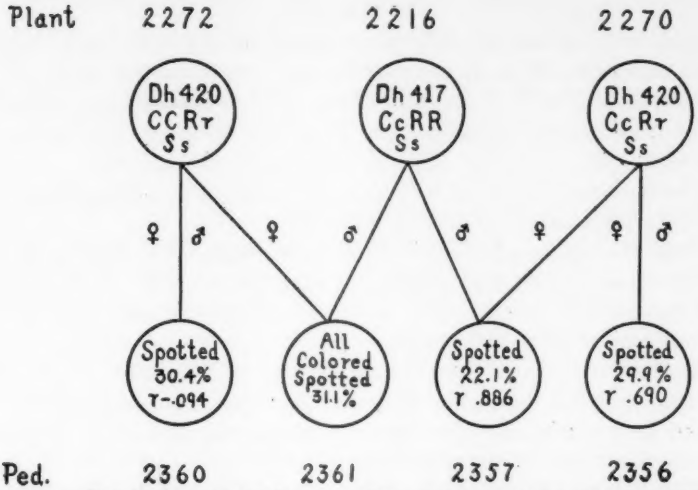


FIGURE 3.—Showing the relationship of ear 2361, an all-colored ear, with 31.1 percent of the seeds spotted. This ear was borne on the same plant, which when self-pollinated produced ear 2360. This ear (2360) had approximately 25 percent of the seeds white. The coefficients of association indicated on the ears 2360, 2357 and 2356 refer to the correlation between endosperm texture and aleurone color.

| ♀ | ♂ *CRS* | *CRs* | *cRS* | *cRs* |
|---|---|---|---|---|
| 7 *CRS* | 7 Self | 7 Self | 7 Self | 7 Self |
| 1 *CRs* | 1 Self | 1 Self | 1 Self | 1 Self |
| 1 *CrS* | 1 Spot | 1 Spot | 1 Spot | 1 Spot |
| 7 *Crs* | 7 Spot | 7 Self | 7 Spot | 7 Self |

DIAGRAM 3.

The present assumption, however, gives a ratio of 46 self-colored to 18 spotted, or 28.1 percent spotted. The observed percentage for the two ears was 29.1 ± .82. The numbers are not sufficient to discriminate between the percentages 29.1 and 28.1, but the facts do not violate the assumption and the observed percentage of spotted seeds is actually lower in the ears with all the seeds colored.

## CONCLUSIONS

Reciprocal crosses between maize plants having white and colored seeds sometimes differ in the distribution of the aleurone color.

The seeds of some of the ears borne on white-seeded plants, if pollinated from colored plants, have the aleurone color distributed in numerous small spots, while the reciprocal crosses are self-colored.

The spotting is assumed to be due to a dominant spotting factor, *S*, which is in the nature of a partial inhibitor.

From the results of this investigation it would seem that the factor which causes spotting can operate only when the factor *R* is heterozygous and furthermore is not present in the female gamete but is introduced into the zygote by the male gamete.

As the male gamete contributes but one nucleus while the female gamete contributes two, the difference between reciprocals may be ascribed to the fact that the female parent contributes twice as much chromatin material as the male parent.

It would appear, therefore, that a single nucleus does not contain enough of the factor *R* to produce a self-colored seed in the presence of the dilution factor *S*.

The percentage of spotted seeds and the fact that spotted seeds are found only on those self-pollinated ears which have no correlation between aleurone color and endosperm texture, has led to the conclusion that there is a coupling or linkage between the factor for aleurone color, *R*, and the factor for spotting, *S*.

It is to be noted further that the gametic ratio in these hybrids is an approximation to a 7:1:1:7 series, equivalent to the location of the two factors on the same chromosome, 12.5 units apart.

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# CHARACTER CHANGES CAUSED BY MUTATION OF AN ENTIRE REGION OF A CHROMOSOME IN DROSOPHILA.[1]

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A female, just hatched, having wings serrated or notched at the end, was found in the purple stock bottle, October 7, 1918. In order to test whether this character was due to a new mutation the female was mated to males from an unrelated stock. The majority of her few $F_1$ daughters had typically "notch" wings quite like the females in the old stocks called notch (see MORGAN and BRIDGES 1916). Later crosses in which larger numbers were obtained always gave notch females, normal females, and normal males in equal numbers, but no notch males, showing that the mutant was a sex-linked dominant, acting in addition as a lethal in the male, precisely like the old notch. It was possible to ascertain that the purple stock had not been contaminated. The character was accordingly considered as due to a reappearance of the old notch gene. Notch was known to have reappeared six times since it first occurred.

In order to find out whether the location of the new notch ("notch$_8$") gene was identical with that of the earlier ones, two cultures were raised in which a red-eyed notch$_8$ female was crossed to males carrying the sex-linked recessive genes eosin (eye color) and crimson (eye color). These genes are located fairly near to the left and to the right respectively of the old notch gene.

When the $F_1$ flies hatched, a paradoxical result was obtained: All the notch$_8$ daughters, instead of being red-eyed as expected, had a yellowish eosin-like eye color. Since the normal-winged daughters did not show this exceptional eye color, the latter could not be due to a duplication of the eosin gene in the X chromosome received from the eosin crimson fathers, and it was at once regarded as probable that the appearance of $F_1$ eosin notch females was due to a "deficiency" (BRIDGES 1917) in the

[1] From the Zoölogical Laboratory of COLUMBIA UNIVERSITY.

X chromosome containing the notch$_8$ gene. If a deficiency, i.e., loss or inactivation, of a piece of the X chromosome carrying the normal allelomorph for eosin were present in the notch$_8$ flies, notch$_8$ females heterozygous for eosin would be somatically eosin, or lighter, with regard to eye color.

An important support for the deficiency-explanation was found in the fact that males which received the notch$_8$ chromosome all died. BRIDGES (1917) had shown that bar-deficiency acted as a lethal for the male. Moreover, notch females from two of the earlier notch stocks, in crosses to males carrying the recessive sex-linked gene for facet (eyes), located near to the left of notch, had given notch-facet females in $F_1$ (METZ and BRIDGES 1917).

When it was taken into consideration that notch$_8$ which had a peculiar effect on eosin analogous to that previously obtained with facet, also agreed with old notches in a series of somatic respects, as will be pointed out later, it was regarded as probable that the character notch itself might be due to deficiency and not to a gene of the common kind. If this were true it was to be expected that the deficiency in the notch$_8$ flies covered a region extending at least from eosin (at 1.1) to the locus of the old notch (at 2.6), a distance of 1.5 units. This was an extraordinarily favorable case for further analysis, since the following mutant genes were present in this region of the X chromosome: White and its 8 allelomorphs, one of which is eosin (at 1.1), facet (at 2.2) and the gene for the dominant sex-linked character abnormal abdomen (at 2.4). Two of these characters, viz., eosin and facet, were in addition sex-limited, both being much more extreme in males than in females.

On the basis of the working hypothesis mentioned, it could be predicted that notch$_8$ females, when crossed to males carrying the recessive sex-linked genes just spoken of, would give $F_1$ notch$_8$ daughters which, in spite of being heterozygous, would show the characters. By crossing notch$_8$ females to males carrying other sex-linked genes to the left and to the right of this region it would be seen in $F_1$ if other loci also were included in the deficient region, and a preliminary measure of the extent of the deficiency could in this way be obtained.

The result of these crosses proved the hypothesis to be correct. White and all its allelomorphs manifested themselves in the heterozygous $F_1$ notch$_8$ females; so also did facet. Abnormal abdomen, being partially dominant, would be expected to appear to some extent anyhow. Special facts with regard to the latter cross will be given later. None of the other

sex-linked genes (those to the left and right) showed in the $F_1$ notch$_8$ females. The gene nearest eosin to the left is the recessive broad (wings), (at 0.4), the one nearest to the right of the old notch is the recessive echinus (eye), (at 5.6). It could accordingly be concluded that the deficient region was not long enough to cover this distance, 5.2 units.

The examination of the $F_1$ notch$_8$ females heterozygous for the allelomorphs of white or for facet or abnormal abdomen revealed a new and important fact. The notch$_8$ flies heterozygous for an allelomorph of white had a lighter eye color than the flies in the corresponding stocks. This exaggerating effect of deficiency was especially striking in notch flies heterozygous for the darker allelomorphs of white, such as cherry, coral or blood. Similarly heterozygous notch$_8$ facet females had much more extreme facet eyes even than the facet males. Also the notch$_8$ females heterozygous for abnormal abdomen had a more marked abnormal abdomen than their heterozygous abnormal-abdomen sisters, though this difference was less pronounced than was the case with regard to facet. This result indicated that the deficiency exaggerates the effect of all the mutant genes present in the corresponding region of the other X chromosome.

To test this point under optimal conditions, deficiency notch$_8$ females heterozygous for white or the allelomorphs of white were back-crossed in pair matings to males from the white or to the corresponding white-allelomorph used in the cross. The not-notch females among the progeny were homozygous for the allelomorph of white used, and direct comparison was made between them and the eye colors modified by notch. The result of this experiment fully confirmed the earlier observation. The notch$_8$ flies always had lighter and more transparent eyes than their homozygous sisters. This effect of deficiency could be detected even in notch flies heterozygous for the lighter eye colors belonging to the white-allelomorph series.

An exaggerating effect of deficiency on other light eye colors was observed in other experiments. When the deficiency notch$_8$ was present in flies homozygous for the sex-linked genes vermilion and garnet, these eye colors were markedly lighter than in the not-notch sisters. Likewise notch$_8$ females homozygous for pink, the gene of which is in the third chromosome, showed a lighter eye color than homozygous pink flies. No similar effect on the darker eye color sepia in the third chromosome could be observed.

From these tests it was seen that deficiency notch$_8$ acted very much

like the factor for white itself. For, not only are compounds of white with its allelomorphs lighter than the later allelomorphs when homozygous, but the gene for white when present in heterozygous condition has also a diluting effect on some other eye colors, such as pink (MORGAN and BRIDGES 1913).

It was found, however, in the course of the above experiment, that deficiency seemed to have an even more pronounced effect than white itself was known to have. To ascertain this point, notch$_8$ females heterozygous for white were back-crossed in pair matings to males carrying the different allelomorphs of white. This would be a decisive experiment since the $F_1$ notch$_8$ daughters would have deficiency in one chromosome and the allelomorph of white in question in the other, while their sisters would have white in one chromosome and the same allelomorph in the other. The comparison could in this way be carried out under absolutely equal conditions, since the flies to be compared were raised in the same culture and derived from the same parents.

The result of this experiment proved the correctness of the above supposition. The deficiency-allelomorph compound was always somewhat lighter and more transparent than the corresponding white-allelomorph compound. Even in the lighter eye colors of the allelomorphic series the females could be separated on the basis of this difference in eye color. Deficiency notch$_8$ acts accordingly as a sub- or infra-white.

The marked exaggerating influence of the deficiency notch$_8$ on the effect of all the mutant genes located in the corresponding region of the other X chromosomes, could hardly be brought into accord with the earlier conception that deficiency represented a physical loss or a total inactivation. It was accordingly regarded highly desirable to be able to compare a notch$_8$ female heterozygous for one of the darker allelomorphs of white with a non-disjunctional XO male carrying the same allelomorph in its X chromosome. Such a male would show the effect, if any, of the total loss, since it has only one X chromosome, the entire Y chromosome being absent. No such effect in the XO males had previously been recorded, but much attention could hardly have been paid to this special point.

By chance a non-disjunctional XO cherry male occurred in a cross between a notch$_8$ female and a cherry abnormal-abdomen male. This male could accordingly be compared with his own notch sisters heterozygous for cherry. That the male in question really was of the above non-disjunctional type could be ascertained by the fact that though tested

with six females, he proved to be sterile, since BRIDGES (1916) has demonstrated that non-disjunctional XO males are always sterile. This XO cherry male had the ordinary dark eye color of regular cherry males, much darker than that of his heterozygous deficiency-cherry sisters.

An analogous exaggerating effect of deficiency was, as mentioned, observed with regard to facet. Heterozygous facet-notch$_8$ females have a rough eye much more extreme than that of facet males, which in turn show the facet character much more pronounced than homozygous facet females. Moreover, the wings of the heterozygous notch-facet females were always very much modified, being constantly very markedly notched at the ends and in addition along the sides, so that the whole wing had a spade-like form. These squared-off wings were in addition extended, forming an angle with each other. When the facet stock was looked over it was observed that facet flies showed a tendency to a slight notching of the ends of the wings, most frequent in the males. The modified wing of heterozygous notch$_8$-facet females was accordingly regarded as an expression of this tendency to notching produced by the ordinary facet gene, exaggerated, in this case, by its combination with the deficiency notch$_8$.

With regard to the notch$_8$ $\times$ abnormal-abdomen crosses it should be noticed that the dominant character abnormal abdomen demands special conditions in the culture bottle to manifest itself (MORGAN 1915). It would be regarded *a priori* as fairly probable that the deficiency, exaggerating the effect of the genes in the opposite region, would cause a dominant character of this type, which had its gene within this region, to manifest itself in $F_1$ notch$_8$ flies heterozygous for abnormal abdomen, even if the culture conditions were not favorable for a general manifestation of the character. This was found, however, not to be the case. Several crosses were made up by mating notch$_8$ females to abnormal-abdomen males in which cultures the latter character failed to show in $F_1$ either in the notch or in the not-notch females. In later experiments, however, where the attempts to create the special conditions necessary for the development of abnormal abdomen were successful, it was found that notch females generally had a more marked abnormal abdomen than their heterozygous, not-notch sisters.

Summing up, we find that the deficiency notch$_8$ resembles the earlier described bar-deficiency in the fact that, when it is present, recessive genes in that region which is homologous to the deficient region manifest themselves when present in heterozygous condition. Like the bar-deficiency, too, this new deficiency also acts as a lethal in the males.

But in addition to allowing them to show, this new deficiency exaggerates the effect of all known mutant genes located in the corresponding region of the opposite X chromosome. This effect was not observed in the case of bar-deficiency.

The analysis of bar-deficiency led to the conclusion that deficiency was due to a physical loss or a total inactivation of an entire region of a chromosome. These alternatives, loss or inactivation, can hardly be maintained as an explanation of the case of deficiency here described. This deficiency is shown to have a striking general effect on all mutant genes in the corresponding region of the other X chromosome. It would be very difficult to conceive that this effect could be caused by a total loss or a complete inactivation, for it has been demonstrated that the total absence of one sex chromosome in a non-disjunctional XO male (which contains not even a Y chromosome) had not the slightest effect upon a mutant character (cherry) which was markedly changed by the deficiency notch$_8$.

Moreover, ordinary males (XY) carrying one of the allelomorphs of white are all, except eosin, of just the same eye color as females homozygous for the same allelomorph.

Thus, if the deficiency were regarded as a total loss or a complete inactivation we should be confronted with the situation that, whereas a single gene in the female gives the lighter eye color seen in the heterozygous notch$_8$ flies, two genes together in the female give an eye color exactly like that of the male carrying one gene. This would be a too strange coincidence when we remember that it applies to six genes of very different grades of eye color.

It has been shown that deficiency notch$_8$ exaggerates the effect of all the known mutant genes located in the corresponding region of the other X chromosome. The visible result is that flies carrying the deficiency in one chromosome and one of these genes in the other are further removed from normal, than is the case in homozygous females or males carrying the same gene.

The question arises accordingly if normal genes in the region opposite to the deficient piece are not also influenced in a similar way.

To some extent this seems to be the case. The series of somatic abnormalities found in the notch$_8$ flies points in this direction. The notch$_8$ flies are different from wild-type flies in the following respects: The wings are irregularly nicked at the ends, and often somewhat extended forming an angle with each other; certain veins are thickened; the eyes

are generally small and show a slight tendency to roughening; the acrostical hairs are irregular in their distribution, not arranged in definite rows as is the case in the wild-type fly; extra scutellar bristles are apt to appear.

It is natural to suppose that these somatic peculiarities are a result of the modification of the effects of one or more of the normal genes in the region opposite to the deficient piece, similar to that which has been demonstrated in the case of the mutant genes. It is superfluous to regard the character notch as due to an independent specific mutant gene contained in or linked to the deficient region.

It would seem probable that many normal genes are contained in such a piece of the X chromosome as that opposite to the deficient region. The fact that more extensive alterations are not caused when the deficient chromosome is present could perhaps be said to point in the direction that the normal genes must have different potencies. The mentioned mutant genes and some of the normal ones in the region opposite are affected, while other normal genes, supposedly present in the same region, are not visibly influenced.

In connection with the description of this case of deficiency the remarkable fact might be recalled that a majority of the known dominant genes in Drosophila are lethal when homozygous, like yellow body color in mice. Notch itself was in fact previously placed in this group (MULLER 1917). The $notch_8$ case is the first in which an explanation of this peculiar relation has been possible. It might very well be that some of the not-sex-linked cases of this type are also due to deficiency. The lethal effect of the genes in homozygous condition is just what was to be expected if this were true. Deficiency may be a more common phenomenon than so far regarded probable. If the region opposite to the deficient piece did not contain mutant genes or normal ones which are modified in their effect in the presence of the deficiency, the deficiencies would not be recorded as such, but only as lethals. Some of the numerous lethals in Drosophila may be due to such deficiencies.

The data obtained concerning the effect of deficiency $notch_8$ on the process of crossing over will not be presented here. It should only be said that no crossing over takes place within the deficient region. The presence of deficiency seems also to influence in a special way the amount of crossing over in the neighborhood of the deficient region. Preliminary linkage tests indicate that the deficient region extends somewhat to the left of eosin and considerably to the right of facet, in fact to within

0.7 unit of echinus, making at total length of about 4.8 units. This indicates that the extent of the notch$_8$ deficiency is so considerable that a cytological examination might throw light on the physical nature of deficiency.

It should be mentioned that of the previously recorded notch mutations two were tested in crosses to facet and proved to be deficiencies for this locus (see above). Five were used in experiments in which they were crossed to white or its allelomorphs, but these genes did not manifest themselves when heterozygous in the notch flies. Deficiencies in this region of the X chromosome may accordingly differ in length and still give the notch character.

The author acknowledges with gratitude his indebtedness to Dr. MORGAN, Dr. BRIDGES and Dr. MULLER for their help during the work.

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# THE INHERITANCE OF THE MUTANT CHARACTER "VORTEX"

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## INTRODUCTORY

There seems to be an impression that the great majority of mutants of Drosophila are comparatively simple in their genetic behavior. Because of their superior usefulness the mutants whose inheritance is clean-cut have been practically the only ones employed in the experiments for the analysis of genetic phenomena. Other mutants occur, and not infrequently, which must be made the objects rather than the tools of investigation. In the following paper is given an account of such a character, "vortex."

## ORIGIN OF THE VORTEX CHARACTER

In looking over the "California wild" stock of *Drosophila melanogaster* in November 1913, an occasional fly was found which showed on the thorax a pair of "rosettes"; that is, in the areas lateral to the dorsocentral bristles the microchaetae or small hairs were arranged in a pair

of whorls. Specimens of this character were noticed on other occasions, but no breeding work was done until August 7, 1916, when a female was found that had in addition to the posterior rosettes an anterior pair. This female gave rise to the stock "vortex," with which the present work has been done.

### DESCRIPTION OF THE VORTEX CHARACTER

In appearance and in degree of development the vortex character is quite variable. In the modal condition (figure 1*b*) two brown-pigmented spots are present, located lateral to and midway between the anterior and posterior dorso-central bristles. The pigment lies in the walls of an indentation or funnel that extends more or less deeply into the thorax. The microchaetae for a considerable area around this focus are arranged in a whorl. Also the dorso-central bristles, especially the anterior pair, are involved in the whorled formation.

In more pronounced specimens (figure 1*c*) these vortices are much more conspicuous and the central funnel is partly evaginated like the

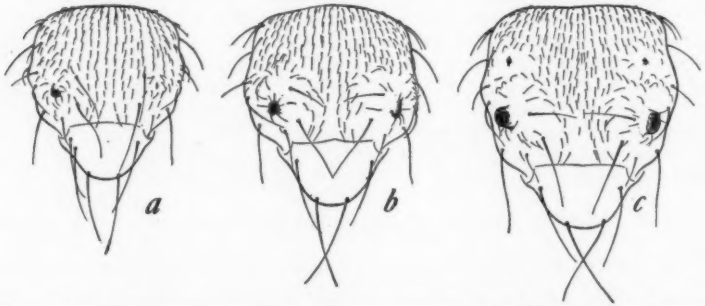


FIGURE 1.—The mutant character vortex. *a* represents a "slight" type (♂) with no anterior vortices; *b* represents the "modal" type (♀) with conspicuous funnel-like vortices; *c* represents an "extreme" type with everted funnels, and an anterior pair of vortices. *a* shows a symmetrical development that is fairly common.

finger of a glove, or even wholly evaginated into a horn-like elevation. In these extreme specimens an anterior pair of whorls is also developed. These anterior vortices are never as pronounced as the posterior ones.

In the "slight" cases (figure 1*a*) only the rosette-like arrangement of the hairs, or only a small brown pigmented depression indicates the character. A very small proportion of the individuals, genetically the same as the others, entirely fail to show the character, and are somatically normal.

All intergrades between this complete absence of the character and its fullest expression are met with in the same culture, although the bulk of the individuals are of the "modal" type.

In general the degree of development is approximately the same on the two sides. But within all grades there may be asymmetrical development of the character, so that the anterior vortex on one side may be absent giving a tri-vortex condition; also uni-vortex individuals are not uncommon.

The character is sex-limited to a considerable degree; i.e., females exhibit a higher grade of the character than do the males. The slight grades are preponderantly males and those few individuals that fail to show the character are practically always males.

The vortex flies do not differ from wild flies in any other respect that we have observed, and they are of good viability.

## THE BIGENIC NATURE OF THE VORTEX CHARACTER

The first test usually made with a mutant character is the determination of the chromosome to which its gene belongs. The most approved method for doing this is to cross the flies in question to flies carrying the dominant characters star and dichaete, star being in the second chromosome (at 0.0) and dichaete in the third (at 11.7). By means of back-cross tests of the $F_1$ star dichaete males it is possible to ascertain whether the gene for the mutation lies in the second or the third chromosome or whether it is independent of both. The star dichaete back-cross method takes advantage of the fact of no crossing over in the male. Thus, if the gene for the mutant in question is in the second chromosome all the back-cross progeny will fall into two classes, namely, those showing star and those showing the mutant, with a total absence of individuals showing both star and the mutant, or, conversely, neither.

Accordingly, the original vortex female was at once out-crossed to a star dichaete male. Apparently this female was non-virgin and had already been fertilized by a brother heterozygous for vortex, since of the $F_1$ offspring, 17 showed the character vortex while the remaining 61 did not show vortex but did show the star and dichaete in all combinations but with some excess in the wild-type class( culture 4955, Aug. 17, 1916).

The fact that criss-cross inheritance was not shown—that among the $F_1$ males none of the stars or dichaetes showed vortex—proved that the character is not sex-linked.

The fortunate presence of the vortex flies in the $F_1$ culture bottle gave immediate materials for making the desired back-cross test. Four back-cross cultures were raised from matings between sister vortex females and $F_1$ males heterozygous for star, dichaete and vortex.

TABLE 1

*$P_1$ mating, star dichaete male by vortex female; back-cross mating, vortex female by $F_1$ star dichaete male.*

| 1916 Aug. 31 | | Vortex | Vortex star | Vortex dichaete | Vortex star dichaete | Wild-type | Star | Dichaete | Star dichaete |
|---|---|---|---|---|---|---|---|---|---|
| 5056 | ♀ | 16 | — | — | — | 2 | 11 | 12 | 16 |
| | ♂ | 6 | — | — | — | 11 | 12 | 12 | 22 |
| 5077 | ♀ | 12 | — | 1 | — | — | 18 | 10 | 21 |
| | ♂ | 6 | — | — | — | 1 | 17 | 5 | 18 |
| 5078 | ♀ | 18 | — | — | — | 27 | 11 | 13 | 26 |
| | ♂ | 15 | — | — | — | 18 | 22 | 10 | 20 |
| 6126 | ♀ | 13 | — | 1 | — | 13 | 2 | 9 | 9 |
| | ♂ | 16 | — | — | — | 7 | 9 | 14 | 20 |
| Total | ♀ | 59 | — | 2 | — | 42 | 42 | 44 | 72 |
| | ♂ | 43 | — | — | — | 37 | 58 | 41 | 80 |
| Grand total | | 102 | — | 2 | — | 79 | 100 | 85 | 152 |

The first point observed was that none of the back-cross star flies showed the vortex character. This linkage between star and vortex indicated that a second-chromosome recessive was essential for the production of vortex. Aside from two female exceptions none of the dichaete flies was vortex; by the same reasoning it is then obvious that the production of vortex depends also upon the action of a third-chromosome recessive gene. That is, the vortex character is the product of the simultaneous action of two independent genes, one in the second and one in the third chromosome.

If the above hypothesis is correct the vortex class should appear as one quarter of the offspring of such a back-cross test. In fact, the vortex individuals totaled 104 out of 520 or 21.2 percent, which is a fairly close approximation to the 25 percent expected. Furthermore, taking account of the fact that there is no crossing over in the male the back-cross flies should be in the ratio 1 vortex: 1 star: 1 dichaete: 1 star dichaete, which is approximated in the observed ratio 102 vortex: 100 star

: 85 dichaete: 152 star dichaete, although the star dichaete class is too large.

There were two further points in which the results failed to agree with the simple explanation thus far suggested. Two dichaete vortex females occurred, which were explained by the assumption that occasionally an individual homozygous for vortex II and heterozygous for vortex III shows the character. Later work has confirmed this hypothesis, for it has shown that the vortex III gene is not strictly recessive in flies homozygous for vortex II. The second point is the occurrence of the large and unexpected class of wild-type flies. An explanation for some of these wild-type flies was obtained when vortex flies were bred together (table 2).

TABLE 2

*Progeny from crosses of vortex female by vortex male.*

| 1916, Sept. 20 | Vortex ♀ | Wild-type ♀ | Vortex ♂ | Wild-type ♂ |
|---|---|---|---|---|
| 5255 | 98 | 1 | 84 | 19 |
| 5271 | 99 | 1 | 95 | 6 |
| 5458 | 12 | — | 10 | — |
| 5466 | 2 | — | 5 | — |
| 5467 | 8 | — | 2 | — |
| 5468 | 10 | — | 2 | — |
| 5469 | 17 | — | 17 | — |
| 5676 | 55 | 1 | 45 | 13 |
| 5701 | 27 | — | 45 | — |
| 5702 | 37 | — | 44 | 5 |
| 5867 | 64 | 2 | 59 | 10 |
| Total | 429 | 5 | 391 | 53 |

In making the counts of table 2 it was apparent that especially in the males a considerable proportion (about 20 percent), showed the character to a very slight extent. It is probable that some of the so-called wild-type flies in the back-cross counts of table 1 would have shown the vortex in this slight form had they been classified with knowledge of this point. However, even with close examination certain flies of the "pure cultures" of table 2 failed to show the character. In this connection an interesting fact was noted; namely, that those few wild-type females which occurred were among the last to hatch. Thus, in cultures 5255, 5271, and 5676 the single wild-type females occurred in the last day's count. A similar phenomenon was noted with regard to the males. The first flies to hatch were all quite extreme vortexes. Somewhat later the

males were less extreme on the average, and an occasional wild-type male occurred. In those cultures in which a large number of wild-type males appeared the majority were in the latest counts.

But that the above points do not entirely explain the back-cross results is apparent when it is realized that in two of the cultures (5078, 6129) there were many wild-type females. It seems probable from later work that in these two cultures the vortex mother was of the type heterozygous only for the vortex III gene, in which case the number of wild-type and of vortex offspring should be about equal.

### THE LOCI OF THE VORTEX GENES

The same mating which gave material for the above back-cross tests of the male, gave an opportunity to test at once the question of the localization of the two genes concerned. By crossing the $F_1$ star dichaete females from the cross of vortex by star dichaete, to vortex males the amount of crossing over between star and the second-chromosome gene, and at the same time the amount of crossing over between dichaete and the third-chromosome gene, could be found. Out of the more than twenty such cultures that were started only three were successful (table 3).

The results of the female back-crosses parallel quite closely those of

TABLE 3

*$P_1$ mating vortex female by star dichaete male; back-cross mating, $F_1$ star dichaete female by vortex male.*[1]

| 1916 Aug. 28 | | $v_o$ | $S$ $v_o$ | $D$ $v_o$ | $S$ $D$ $v_o$ | + | $S$ | $D$ | $S$ $D$ |
|---|---|---|---|---|---|---|---|---|---|
| 5047 | ♀ | 16 | — | 8 | 1 | 2 | 15 | 7 | 11 |
| | ♂ | 6 | — | 1 | — | 2 | 15 | 7 | 11 |
| 5254 | ♀ | 3 | — | 2 | — | 1 | 10 | — | 9 |
| | ♂ | 8 | — | 1 | — | 2 | 3 | 4 | 6 |
| 5866 | ♀ | 11 | — | 2 | — | 2 | 5 | 17 | 13 |
| | ♂ | 12 | 1 | 6 | — | — | 6 | 13 | 22 |
| Total | ♀ | 30 | — | 12 | 1 | 5 | 30 | 24 | 33 |
| | ♂ | 26 | 1 | 8 | — | 4 | 16 | 29 | 42 |
| Grand total | | 56 | 1 | 20 | 1 | 9 | 46 | 53 | 75 |

[1] The symbol for the dominant character star is the capital letter $S$; that for the designated $v_o$; the vortex gene situated in the second chromosome is $v_{oII}$, that in the third is $v_{oIII}$. The + sign is read "wild-type."

the male back-cross of table 1, except that apparent crossovers occurred. The small number of wild-type flies, especially males, in these cultures indicates that there was very slight changing over of vortex into wild-type, and it is probable that these wild-type flies, certainly the females, represent crossing over between star and vortex II. A calculation of the location of the second-chromosome vortex gene gave a star vortex crossover value of about 10.

There was uncertainty with respect to the dichaete vortex class, since in the male tests of table 1, where there was no crossing over, there had appeared two such dichaete vortex females. A calculation made on the uncertain basis that the dichaete vortex flies were crossovers gave a total of 21 vortex dichaete among the 78 vortex or 27 percent of crossing over. It was accordingly decided as possible that the third-chromosome gene for vortex lies to the right of dichaete at a position near to the center of the third chromosome.

It had been hoped to get far more adequate data with respect to the location of the second- and third-chromosome genes. But an unexpected obstacle presented itself. It was only with the greatest difficulty that the cultures involving vortex could be reared. The back-cross tests of the female were started on a large scale but of the more than twenty cultures all but three proved sterile, and these three produced relatively few flies. The same low productivity had been apparent in some of the pair cultures of vortex by vortex of table 2.

From the preceding considerations it was apparent that several points were capable of further elucidation. The occurrence of the class of wild-type flies in the back-cross tests of the male (table 1) was not accounted for beyond question. The appearance of dichaete vortex flies, which simulated crossovers in the male back-cross test, had received an explanation requiring experimental tests. It seemed possible that sterility was in some way connected with the above aberrations. Furthermore, a case ("pale") had arisen the characteristics of which gave a suggestive parallelism with the vortex case, namely, simultaneous linkage to both the second and the third chromosome, the appearance of unexpected classes, and of lethal effect. The analysis of that case had led to the hypothesis that a piece of the second chromosome had been removed and had been attached to the middle of the third chromosome. The removal of the piece of the second chromosome (deficiency) gave the effect of a lethal located in the second chromosome. The attachment of that piece to the third chromosome (duplication) explained the linkage of the contained

genes to the third chromosome genes. The place of attachment of this "transposed" piece was apparently at the middle of the third chromosome, which is the place of the spindle-fiber attachment. If the simultaneous linkage of vortex to the second and to the third chromosome was based on some such "transposition" then the transposed piece should likewise be attached to the spindle fibre and the linkage of vortex should correspond to a locus at the middle of the third chromosome. The preliminary calculation had suggested that this indeed was the case since there seemed about 27 percent of crossing over between dichaete and vortex, dichaete being known to be some 25 units from the center of the chromosome. A test of the above points demanded first of all a repetition of the original experiments on a larger scale and with close attention to the questionable features.

### TESTS OF THE WILD-TYPE FLIES OF VORTEX STOCK

The first point tested was the assumption that the wild-type flies that occurred in the stock of vortex were simply fluctuants and were of the same genetic constitution as those flies which showed the character. A pair of such wild-type flies (M251) gave 62 vortex individuals and only two wild-type individuals, which were males. This was an entirely regular result comparable with the progeny given by vortex pairs. A second pair (M312) gave 71 females all of which were vortex and only four wild-type males among the 65 males of the culture. A pair of extreme vortex individuals mated at the same time gave 91 vortex and 13 wild-type individuals (M236). From these tests it is apparent that flies of the stock may give the same results irrespective of their grade, that is, of their somatic appearance.

### REPETITION OF THE MALE BACK-CROSS TEST WITH STAR DICHAETE

The second test was a repetition of the male back-cross which had given the numerous wild-type flies. Ten such cultures were raised with no sterility (table 4).

During the classification of the flies of table 4 particular attention was paid to the classes which had caused confusion before, namely the wild-type and the dichaete vortex classes, in order that none of the flies classed as wild-type should show vortex even slightly, and likewise that all of the dichaete flies which showed the vortex might be separated out.

The first point which appeared in the new cultures was that the number of wild-type flies in no case exceeded the number which could read-

TABLE 4

*$P_1$ mating, vortex female by star dichaete male; back-cross mating, vortex female by $F_1$ star dichaete male.*

| 1918 July 9 | | $v_0$ | $S$ $v_0$ | $D$ $v_0$ | $S$ $D$ $v_0$ | + | $S$ | $D$ | $S$ $D$ |
|---|---|---|---|---|---|---|---|---|---|
| M337 | ♀ | 21 | — | — | — | — | 20 | 18 | 39 |
| | ♂ | 20 | — | — | — | 3 | 21 | 16 | 28 |
| M338 | ♀ | 18 | — | — | — | — | 21 | 23 | 15 |
| | ♂ | 10 | — | — | — | 9 | 10 | 11 | 12 |
| M339 | ♀ | 28 | — | 7 | — | — | 26 | 17 | 22 |
| | ♂ | 12 | — | — | — | 1 | 35 | 25 | 24 |
| M340 | ♀ | 15 | — | — | — | — | 23 | 18 | 30 |
| | ♂ | 21 | — | — | — | 1 | 18 | 22 | 23 |
| M341 | ♀ | 21 | — | 3 | — | — | 31 | 14 | 20 |
| | ♂ | 20 | — | — | — | 2 | 18 | 23 | 26 |
| M347 | ♀ | 29 | — | 3 | — | — | 30 | 19 | 28 |
| | ♂ | 25 | — | — | — | 2 | 21 | 22 | 26 |
| M348 | ♀ | 26 | — | 3 | — | — | 21 | 15 | 19 |
| | ♂ | 23 | — | — | — | 1 | 34 | 20 | 29 |
| M350 | ♀ | 12 | — | 1 | — | — | 14 | 13 | 16 |
| | ♂ | 10 | — | — | — | — | 15 | 11 | 18 |
| M354 | ♀ | 18 | — | 8 | — | — | 17 | 10 | 12 |
| | ♂ | 17 | — | — | — | 3 | 15 | 14 | 22 |
| M356 | ♀ | 26 | — | 2 | — | — | 17 | 20 | 26 |
| | ♂ | 20 | — | — | — | 1 | 21 | 20 | 27 |
| Total | ♀ | 214 | — | 27 | — | — | 220 | 167 | 227 |
| | ♂ | 178 | — | — | — | 23 | 208 | 184 | 235 |
| Grand total | | 392 | — | 27 | — | 23 | 428 | 351 | 462 |

ily be explained by the amount of overlap of vortex into normal. This wild-type class was further characterized by being entirely confined to the males, not a single wild-type female having occurred. As had been discovered in the case of the wild-type fluctuants in the vortex stock these wild-type males were in general restricted to the late counts.

The sums of the wild-type and the vortex flies gave a class of the same size as each of the other expected classes and not a large excess as had been the case in the two exceptional cultures of table 1. Definite proof in the case of two of the wild-type flies that they were genetically vortex was furnished by crossing them to vortex females from stock. One of the tested males gave 62 vortex females, 79 vortex males and only three wild-types which were males (M431). The other gave 32 vortex females and 21 vortex males with no wild-type flies (M440).

The apparent crossover class of dichaete vortex reappeared in the new

experiment, but was entirely confined to the females. It had been suggested on the basis of the former experiments that these dichaete vortex flies were not homozygous for both vortex genes, but were homozygous for the second-chromosome gene and only heterozygous for the third-chromosome gene. If this were true such flies should give, when tested by homozygous vortex males, about half of the offspring vortex and half not vortex. Were they really homozygous for both genes, almost all of the offspring ought to be vortex as in a stock culture. One such vortex dichaete female (from M354) was accordingly tested by crossing to vortex males from stock. The offspring (M396) were: $v_0$♀ 29, +♀ 0, $D$♀ 41, $Dv_0$♀ 1, $v_0$♂ 26, +♂ 6, $D$♂ 39, $Dv_0$♂ 0. That is, the dichaete-bearing third chromosome did not carry the vortex III gene, and approximately half of the flies were vortex instead of nearly all being vortex. This result proved that it is possible for a female only heterozygous for the third-chromosome vortex gene to show the character when homozygous for the second-chromosome gene. Furthermore the single dichaete vortex female which occurred in the above test culture was of the same constitution as her mother as was proved by the progeny obtained by crossing her to a vortex male from stock. The progeny were: $v_0$♀ 76, +♀ 0, $D$♀ 70, $Dv_0$♀ 15, $v_0$♂ 83, +♂ 3, $D$♂ 83, $Dv_0$♂ 0, among which progeny the vortex constituted 174 out of 330 flies.

Since the suggested explanation has proved to be correct, an interesting comparison between this dichaete vortex and the wild-type class presents itself. All the wild-type flies were male, but the dichaete vortex flies were without exception female. While this is apparently an inverse relation it is in reality an expression of a single phenomenon—the partial sex-limitation of the character. This sex-limitation permits a readier and more marked expression of the character in the female than in the male, both in the double homozygous condition and also in the special case of the heterozygote just considered.

### LOCATION OF THE VORTEX GENES THROUGH LINKAGE TESTS WITH STAR AND DICHAETE

The first experiment to locate the vortex genes more accurately was a repetition of the female back-cross test with star and dichaete (table 5).

The results to be expected from the female back-cross are much more complex than those that form the male tests; in addition to the changing over of vortex into wild-type (♂♂) and the presence of supernumerary vortexes of the heterozygous type (♀♀), in the case of the female test

TABLE 5

*$P_1$, vortex ♀ × star dichaete ♂; $F_1$ star dichaete ♀ × vortex ♂.*

| 1918 July 14 | | $v_o$ | $S$ $v_o$ | $D$ $v_o$ | $S$ $D$ $v_o$ | + | $S$ | $D$ | $S$ $D$ |
|---|---|---|---|---|---|---|---|---|---|
| M359 | ♀ | 34 | 10 | 7 | 1 | 2 | 30 | 26 | 35 |
| | ♂ | 36 | 3 | — | — | 5 | 32 | 32 | 27 |
| M366 | ♀ | 34 | 1 | 1 | — | 5 | 38 | 47 | 44 |
| | ♂ | 28 | 4 | — | — | 8 | 37 | 48 | 43 |
| M367 | ♀ | 36 | 5 | 13 | 2 | 7 | 28 | 39 | 38 |
| | ♂ | 40 | 2 | — | — | 3 | 33 | 48 | 59 |
| M368 | ♀ | 40 | 3 | 8 | 1 | 3 | 41 | 29 | 52 |
| | ♂ | 38 | 2 | — | — | 8 | 44 | 60 | 37 |
| M397 | ♂ | 27 | 2 | 10 | 2 | 7 | 26 | 40 | 35 |
| | ♀ | 37 | 3 | — | — | 5 | 27 | 39 | 32 |
| M398 | ♀ | 16 | 1 | 7 | 5 | 2 | 11 | 15 | 24 |
| | ♂ | 16 | 2 | — | — | 2 | 17 | 16 | 13 |
| M399 | ♀ | 44 | — | 10 | 2 | 2 | 36 | 29 | 41 |
| | ♂ | 31 | 7 | — | — | 8 | 37 | 42 | 35 |
| M400 | ♀ | 32 | 5 | 11 | — | 5 | 39 | 26 | 35 |
| | ♂ | 35 | 2 | — | — | 9 | 36 | 36 | 39 |
| M401 | ♀ | 22 | 3 | 6 | 1 | 4 | 17 | 26 | 27 |
| | ♂ | 27 | 1 | — | — | 3 | 13 | 27 | 20 |
| M402 | ♀ | 31 | 2 | 1 | — | 2 | 26 | 25 | 33 |
| | ♂ | 38 | 7 | — | — | 5 | 27 | 27 | 31 |
| M425 | ♀ | 22 | 2 | — | — | 2 | 13 | 11 | 26 |
| | ♂ | 8 | 1 | — | — | 1 | 13 | 13 | 19 |
| M426 | ♀ | 23 | 4 | 1 | — | 2 | 29 | 32 | 34 |
| | ♂ | 37 | 5 | — | — | 6 | 35 | 45 | 39 |
| M427 | ♀ | 42 | 2 | — | — | 2 | 36 | 30 | 41 |
| | ♂ | 29 | 2 | — | — | 3 | 37 | 36 | 47 |
| M428 | ♀ | 31 | — | — | 1 | 4 | 45 | 29 | 40 |
| | ♂ | 41 | 4 | — | — | 8 | 40 | 35 | 32 |
| M429 | ♀ | 37 | 4 | 2 | — | 3 | 28 | 41 | 36 |
| | ♂ | 19 | 4 | — | — | 4 | 33 | 57 | 37 |
| Total | ♀ | 471 | 44 | 77 | 15 | 52 | 443 | 445 | 541 |
| | ♂ | 460 | 49 | — | — | 78 | 461 | 555 | 510 |
| Grand total | | 931 | 93 | 77 | 15 | 130 | 904 | 1000 | 1051 |

crossing over gives classes identical in appearance but different in their genetic origin. Thus the non-vortex classes are each composed, theoretically, of progeny from three sources according to whether they represent crossing over in the second, the third, or in both the second and the third chromosome, as may be seen from table 6.

A fortunate simplification of this problem is obtained from a con-

TABLE 6

*Classes of eggs produced by a female of the type,* $\frac{S}{v_{o\,II}}\ \frac{D}{v_{o\,III}}$ *, and of offspring when such eggs are fertilized by sperm of a vortex male.*

| Non-crossover (A) | | | | Crossover in II (B) | | | | Crossover in III (C) | | | | Crossover in II and III (D) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\frac{S}{D}$ | $\frac{v_{o\,II}}{v_{o\,III}}$ | $\frac{S_o}{v_{o\,III}}$ | $\frac{v_{o\,II}}{D}$ | $\frac{Sv_{o\,II}}{D}$ | $\frac{+}{v_{o\,III}}$ | $\frac{Sv_{o\,II}}{v_{o\,III}}$ | $\frac{+}{D}$ | $\frac{S}{Dv_{o\,III}}$ | $\frac{v_{o\,II}}{+}$ | $\frac{S}{+}$ | $\frac{v_{o\,II}}{Dv_{o\,III}}$ | $\frac{Sv_{o\,II}}{Dv_{o\,III}}$ | $\frac{+}{+}$ | $\frac{Sv_{o\,II}}{+}$ | $\frac{+}{Dv_{o\,III}}$ |
| $\frac{S}{D}$ | $\frac{v_o}{}$ | $\frac{S}{}$ | $\frac{D}{}$ | $\frac{S}{D}$ | $\frac{+}{}$ | $\frac{S}{v_o}$ | $\frac{D}{}$ | $\frac{S}{D}$ | $\frac{}{+}$ | $\frac{}{S}$ | $\frac{D}{v_o}$ | $\frac{S}{D}$ $v_o$ | $\frac{+}{+}$ | $\frac{S}{}$ | $\frac{D}{}$ |

| | | ♀♀ | ♂♂ |
|---|---|---|---|
| Vortex | = A = | 471 | 460 |
| Star vortex | = B = | 44 | 49 |
| Dichaete vortex | = C = | 77 | — |
| Star dichaete vortex | = D = | 15 | — |

| | | ♀♀ | ♂♂ |
|---|---|---|---|
| Wild-type | = B+C+D = | 52 | 78 |
| Star | = A+C+D = | 443 | 461 |
| Dichaete | = A+B+D = | 445 | 555 |
| Star dichaete | = A+B+C = | 541 | 510 |

sideration of the dichaete and the star dichaete classes. Among the males not a single vortex individual of these classes appeared. Since the changing over of genetically vortex into somatically not-vortex is very slight throughout this experiment, this complete absence of males of these classes means that none or only a negligible amount of crossing over occurred between dichaete and the third-chromosome vortex gene.

We must omit from our calculation of the amount of crossing over between dichaete and vortex III the data from the females, since in the females it is known that individuals simply heterozygous for vortex III $\left(\frac{v_{o\,II}}{v_{o\,II}}\ \frac{v_{o\,III}}{+}\right)$ in some cases show the vortex character. In fact if crossing over between dichaete and vortex III is as rare as is indicated by the male data then all or practically all of the dichaete vortex and star dichaete vortex females were of this heterozygous type. Two of these dichaete vortex and two star dichaete vortex females were tested by crossing to vortex males, and in all cases they proved to be of the heterozygous type (tables 7 and 8).

The complete absence of dichaete vortex males in table 5 is in contrast with their occurrence in the similar experiment of table 2. Instead of quite free crossing over between dichaete and vortex III, as at first supposed to be the case, there is no good evidence of any crossing over at all between them. The dichaete vortex males of table 2 must then have been due to another cause, and certain similar results, to be described in a later section,

TABLE 7

*Tests by vortex males of dichaete vortex females from the female back-crosses of star dichaete by vortex.*

| | | $v_0$ | + | $D$ | $Dv_0$ |
|---|---|---|---|---|---|
| M445 (ex 397) | ♀ | 60 | — | 38 | 32 |
| | ♂ | 68 | — | 87 | — |
| M446 (ex 397) | ♀ | 78 | — | 45 | 30 |
| | ♂ | 88 | 1 | 93 | 1 |

TABLE 8

*Tests by vortex males of star dichaete vortex females from the female back-crosses of star dichaete by vortex.*

| | | $v_0$ | $Sv_0$ | $Dv_0$ | $SDv_0$ | + | $S$ | $D$ | $SD$ |
|---|---|---|---|---|---|---|---|---|---|
| M447 (ex 398) | ♀ | 51 | 41 | 2 | 3 | — | — | 30 | 27 |
| | ♂ | 37 | 40 | — | — | — | — | 32 | 31 |
| M462 (ex 398) | ♀ | 29 | 16 | 9 | 10 | — | — | 16 | 14 |
| | ♂ | 26 | 28 | — | — | — | — | 31 | 23 |

will make it apparent that this cause may have been an additional semi-dominant modifier.

With respect to the linkage between star and the second-chromosome vortex gene, the crossover classes are star vortex and wild-type. When there is considerably more changing over in the male classes than occurred in this experiment there is still no changing over in the females. Four of the wild-type females were tested, and as expected, in no case were they vortexes that had changed over (table 9, cultures 412, 438, 448, 454). The 52 wild-type females of table 5 are therefore all to be considered as true crossovers between star and vortex II. The non-crossover class which corresponds to this wild-type crossover class is the star class of 443 females. Star vortex (44) and vortex (471) are complementary crossover and corresponding non-crossover classes.

Because of the probability of a slight amount of changing over among the males the wild-type males (78) can not be used without correction, which is here of doubtful validity. Of three such wild-type males tested, one was a true wild-type crossover, but the other two were changed over vortex non-crossovers (table 9, cultures M437, M452 and M460).

Males do not show vortex unless they are homozygous for both genes or contain an additional modifier, and all vortex males of table 5 can therefore be used in the calculation. The star vortex class (49) is the

TABLE 9

*Tests of wild-type flies from table 5 by out-crosses to vortex.*

| No. | From | Vortex ♀ | Wild-type ♀ | Vortex ♂ | Wild-type |
|---|---|---|---|---|---|
| M412 (♀) | M367 | 24 | 16 | 23 | 25 |
| M438 (♀) | M367 | 92 | 113 | 109 | 101 |
| M448 (♀) | M397 | 84 | 76 | 79 | 93 |
| M454 (♀) | M400 | 80 | 72 | 82 | 73 |
| M437 (♂) | M366 | 5 | 9 | 13 | 20 |
| M452 (♂) | M402 | 136 | — | 104 | 20 |
| M460 (♂) | M402 | 84 | — | 42 | 24 |

crossover and the vortex class is the corresponding non-crossover (460). The total number of crossovers available is 145 (52 + 44 + 49), and the corresponding total of non-crossovers is 1374. The percentage of crossing over between star and vortex II is therefore 9.5. The locus of star is so far to the left in the second chromosome that with a distance of nearly ten units between the star and the vortex loci it seemed far more probable that the locus of vortex II is to the right of star.

### THE LOCALIZATION OF VORTEX II BY AID OF STREAK

If, as calculated, the locus of vortex II is about ten units to the right of star then the position of the gene could be more accurately obtained by means of the linkage relations of vortex with the dominant mutant streak. The locus of streak was known to be at about 14.7 units to the right of star, although the data on which that location was based was rather meager in amount. The locus of vortex II was therefore considered to be about five units to the left of that of streak. The most advantageous type of back-cross is that known as "alternated" in which the middle mutant gene is in one chromosome and the two end genes in the other $\left(\frac{S \quad S_k}{v_o}\right)$. In order to obtain heterozygous females of the required type a crossover star vortex male was taken from the previous experiment and crossed to streak females from stock (M439). The $F_1$ star streak females were then back-crossed by vortex males (M487). The small proportion of star streak crossovers which occurred among the back-cross offspring were of two types, half were only heterozygous for vortex III while the remaining half were of the desired homozygous type. To eliminate all doubt as to the constitution of the flies used some of the star streak crossovers were tested individually by mat-

ing to vortex females. One of these cultures (M520—$SS_k$ ♀ 69, ♂ 79; $v_o$ ♀ 74, ♂ 76; + ♂ 1) of which the father proved to have been homozygous for vortex III gave many star streak offspring, all of which were homozygous for vortex III and heterozygous for vortex II. Fifteen cultures were raised from star streak females of the above constitution (table 10).

TABLE 10

*Back-cross tests of* $\frac{S \quad S_k}{v_o \text{II}} \quad \frac{v_o \text{III}}{v_o \text{III}}$ ♀ *by vortex* ♂.

| 1918 Oct. 14 | $S$ $S_k$ | $v_o$ | $S$ $v_o$ | $S_k$ | $S$ | $v_o$ $S_k$ | $S$ $S_k$ $v_o$ | + |
|---|---|---|---|---|---|---|---|---|
| M572 ♀ | 30 | 28 | 4 | 2 | — | — | — | — |
| ♂ | 25 | 26 | 2 | 3 | 1 | — | — | 2 |
| M573 ♀ | 23 | 23 | 1 | 2 | — | — | — | — |
| ♂ | 21 | 14 | 3 | 1 | 2 | — | — | — |
| M574 ♀ | 37 | 50 | 3 | 9 | 1 | — | — | — |
| ♂ | 57 | 42 | 3 | 5 | 3 | — | — | 2 |
| M575 ♀ | 40 | 36 | 8 | 9 | 1 | — | — | — |
| ♂ | 44 | 46 | 2 | 7 | 3 | — | — | 6 |
| M578 ♀ | 50 | 53 | 6 | 10 | 2 | — | — | — |
| ♂ | 42 | 65 | 3 | 5 | 5 | — | — | 7 |
| M579 ♀ | 54 | 61 | 10 | 7 | 2 | — | — | — |
| ♂ | 50 | 48 | 4 | 8 | 3 | — | — | 8 |
| M586 ♀ | 41 | 41 | 6 | 4 | — | — | — | — |
| ♂ | 34 | 24 | 5 | 2 | — | — | — | 1 |
| M587 ♀ | 45 | 55 | 9 | 6 | 3 | — | — | — |
| ♂ | 41 | 45 | 5 | 8 | 4 | — | — | 8 |
| M588 ♀ | 36 | 40 | 2 | 5 | 3 | 1 | — | — |
| ♂ | 43 | 33 | 7 | 6 | 2 | — | — | 2 |
| M589 ♀ | 22 | 31 | 4 | 6 | — | — | — | — |
| ♂ | 22 | 31 | 3 | 5 | 2 | — | — | 1 |
| M590 ♀ | 40 | 43 | 4 | 4 | 2 | — | — | 1 |
| ♂ | 44 | 40 | 3 | 10 | 4 | — | — | 1 |
| M592 ♀ | 35 | 32 | 6 | 4 | 2 | — | — | — |
| ♂ | 38 | 32 | 2 | 7 | 4 | — | — | 13 |
| M593 ♀ | 43 | 38 | 2 | 6 | — | — | — | — |
| ♂ | 23 | 26 | 2 | 2 | 2 | — | — | — |
| M594 ♀ | 32 | 34 | 2 | 6 | 3 | — | — | — |
| ♂ | 30 | 40 | 5 | 6 | 3 | — | — | 10 |
| M610 ♀ | 28 | 44 | 3 | 8 | 1 | 2 | — | — |
| ♂ | 27 | 26 | 5 | 14 | — | — | — | 4 |
| Totals ♀ | 556 | 609 | 70 | 88 | 19 | 3 | — | 1 |
| ♂ | 541 | 538 | 54 | 89 | 38 | — | — | 65 |
| Grand totals | 1097 | 1147 | 124 | 177 | 57 | 3 | — | 66 |

The classification of the experiment of table 10 was safeguarded by isolating all doubtful flies for at least five days until the pigment of the streak and of the vortex characters was fully developed and until the bubbles (to be mentioned later) characteristic of streak become pronounced. In certain cases the further precaution of actual test matings was taken so that the separations as recorded in table 10 can be regarded as complete. Three males from M593 that were regarded as possible streak were tested but proved to be non-streak. The same result was obtained from tests of several of the vortex males wherever there was suspicion that they might be streak.

Before making the calculations of the amount of crossing over it is necessary to consider the changed-over classes. Extensive experiments involving this region of the second chromosome have shown that the amount of double crossing over within this distance of fifteen units is practically zero, so that there should be no wild-type class. It is doubtful whether the one wild-type female which occurred was such a double crossover or was a changed-over vortex. It seems more probable that she was a vortex female since she occurred in the last count of the culture. We may therefore add this one to the 609 vortex females. Likewise the 65 wild-type males (four of which were tested and proved to be vortex genetically) are to be added to the 538 vortex males bringing the number up to 603 which is then equal to the number of females. Among the males 10.8 percent of the vortex class changed over. If this same proportion of star vortex males changed over then six males should be transferred from the star class to the star vortex class, reducing the star class to 32 and increasing the star vortex to 60.

While the counts of table 10 were being made a striking fact was observed, namely, that the streak vortex class was practically non-existent, although it had been expected to be as large as the star class. Furthermore the vortex present in the three streak vortex flies recorded as such in table 10 was of a different type from the ordinary vortex, being developed only in the anterior pair of vortices as very slight depressions with little pigment and no whorling of the hairs. Morphologically there seemed some slight reason why the presence of the streak character should interfere with the development of the vortex character. The thorax of streak flies is markedly altered, especially with regard to the musculature, which is largely replaced by large bubbles. In fact this character of the thorax is the clearest one for classification. It is to be noticed that the anterior pair of vortices would

most often escape the interference by these alterations since the anterior pair of vortices is broadly separated laterally, while the center of the streak disturbance is median and posterior.

Tests, that will be described in a later section, were carried out with these particular streak vortex flies and these tests showed that there was probably present a modifier which favored the development of a vortex among streak flies of these cultures and their descendants.

Not only was the streak vortex class unduly diminished, but correspondingly the streak class was unexpectedly large, being 177 flies while its complementary class star vortex was only 130 flies (corrected for changing over). If the streak vortex flies were included with the streak then we should expect that the number of streak and streak vortex flies should be equal to the sum of the star and the star vortex flies. This is found to be the case, since the sum of the streak flies is 180, while the sum of the star flies is 181. There are two ways of calculating how many flies should be removed from the streak class and added to the streak vortex class. The class of streak vortex should be equal to the complementary class star which is 51 (corrected). This required the transference of 48 flies from the streak to the streak vortex class. The other method is to reduce the size of the streak class to that of its complement (130). This would require the transference of 50 flies from the streak to the streak vortex class. Since these two methods agree the corrected classes may be accepted as 129 streak and 51 streak vortex. The final corrected classes stand as in table 11.

TABLE 11

*The classes of table 9 corrected for changing over and for interference by streak.*

| $\frac{S}{S_k}$ | $v_o$ | $\frac{S}{v_o}$ | $S_k$ | $S$ | $\frac{v_o}{S_k}$ | $\frac{S}{\frac{v_o}{S_k}}$ | + |
|---|---|---|---|---|---|---|---|
| 1097 | 1213 | 129 | 130 | 51 | 51 | — | — |

On this basis there was 9.7 percent of crossing over between star and vortex, which is in agreement with the value (9.5) obtained from the star dichaete vortex female back-cross tests of table 5. There was 3.8 percent of crossing over between vortex and streak, which is slightly less than that previously calculated from data less extensive. The locus of streak on the basis of the entire data is at about 13.7 units to the right of star.

### THE STREAK VORTEX MODIFIER

One of the two exceptional streak vortex flies which occurred in culture 610 was out-crossed to vortex males and gave a considerable proportion (about a quarter) of streak vortex flies of this new type (culture 694, table 12). A streak vortex male from among this progeny out-crossed to vortex females from stock likewise gave this type of streak vortex in the same proportion (M782). This stock has been continued for several generations and gives analogous results. The proportion of streak flies showing vortex in these two cultures and the line descended from one of them is exceptional, since in the other cultures the

TABLE 12
*Selection for vortex streak (new type).*

| No. | Parentage | | $S_k$ | $\frac{v_o}{S_k}$ | $v_o$ | + |
|---|---|---|---|---|---|---|
| M694 | $v_o S_k$ ♀ ex 619 × $v_o$ ♂ | ♀ | 23 | 17 | 40 | — |
| | | ♂ | 19 | 6 | 44 | 8 |
| M812 | $v_o S_k$ ♂ ex 694 × $v_o$ ♀ | ♀ | 31 | 8 | 40 | 2 |
| | | ♂ | 17 | 2 | 36 | 7 |

crossover streak flies which are genetically homozygous for vortex fail to show the vortex character. The new condition which has arisen is probably due to a mutant modifier which has the effect of causing the vortex in streak flies to develop but to develop as a new somatic type. Since this type occurred in two cultures and in out-crossed cultures of their descendants, the modifying gene is a dominant. In the mother of culture 610 this dominant modifier was present in the star streak chromosome not far from streak and probably to the right. When crossing over occurred between vortex II and streak, the streak individuals received the modifier and were then better able to show vortex, but in a modified type. This accounts for the individuals in cultures 610 and 588. When a vortex streak crossover female from 610 was out-crossed, most of the streak descendants should be of the same constitution as the mother, that is, homozygous for vortex II and vortex III and heterozygous for streak and for the dominant modifier. The result showed that only about a quarter of the streak flies developed the new type of vortex. It might have been supposed that crossing over between streak and the modifier had reduced the number of flies containing the modifier and hence showing the new vortex. But in the next generation a

male of this constitution was out-crossed and results similar to those of the female out-cross were obtained. Since there is no crossing over in the male the similarity of the female and male out-crosses shows that in the female likewise there was probably little crossing over between streak and the modifier. It is evident then that this modifier is able to bring the new vortex to expression in only about a quarter of the flies of the given constitution.

There are many other cases known in which flies of a given constitution may or may not show a certain character. The determining factor is presumably environmental, and has been proved to be such in several of the cases.

## FURTHER TESTS OF THE POSITION OF VORTEX III

The other experiments had indicated that the position of vortex III was very close to that of dichaete. In fact no certain crossover had been obtained between these two loci. It was thought advisable to get more extensive data on this point in the hope of finding on which side of dichaete the locus of vortex III is situated. Such an experiment would require the simultaneous use of two known loci in the third chromosome. The stock containing the two dominants dichaete and hairless offered the quickest and most convenient method of obtaining such information. Accordingly a vortex male was crossed to a dichaete hairless female and the $F_1$ dichaete hairless females heterozygous for vortex II and for vortex III were tested by vortex male from stock (table 13(A)). As in the previous experiment, females that were not homozygous for vortex III showed the vortex character occasionally. Thus among the dichaete females of table 13 (*A*) 16 showed vortex slightly. Tests of one of the vortex dichaete hairless females showed that it was the supposed heterozygous type (table 13 (*C*), culture M450). Such vortex dichaete flies likewise occurred in two parallel tests of $F_1$ males, and since no crossing over occurs in the males the vortex dichaete flies are clearly of the heterozygous vortex III type (table 13 (*B*)). For this reason it is only among the dichaete males of table 13 (*A*) that real crossing over could be detected. No such dichaete vortex males occurred, which confirms the closeness of vortex III to dichaete, but fails to show the relative order. On the other hand the crossing over between vortex and hairless was of the amount (20.4) to be expected from the known normal distance between dichaete and hairless.

TABLE 13

(*A*) *Back-cross tests of* $\frac{+}{v_0 II} \; \frac{D \;\; H}{v_0 III}$ *female by vortex male.*

| 1918 July 24 | | $v_0$ | $v_0$ | $v_0$ $H$ | $D$ $v_0$ $H$ | + | $D$ | $H$ | $D$ $H$ |
|---|---|---|---|---|---|---|---|---|---|
| M389 | ♀ | 22 | — | 5 | 3 | 21 | 11 | 5 | 43 |
| | ♂ | 12 | — | 4 | — | 37 | 16 | 9 | 48 |
| M390 | ♀ | 27 | — | 5 | 1 | 36 | 11 | 10 | 37 |
| | ♂ | 21 | — | 3 | — | 57 | 9 | 8 | 62 |
| M421 | ♀ | 29 | — | 11 | 7 | 41 | 27 | 15 | 61 |
| | ♂ | 24 | — | 7 | — | 40 | 20 | 13 | 72 |
| M422 | ♀ | 41 | — | 4 | 2 | 38 | 16 | 8 | 74 |
| | ♂ | 30 | — | 12 | — | 45 | 24 | 7 | 78 |
| M423 | ♀ | 31 | — | 11 | 3 | 40 | 23 | 8 | 62 |
| | ♂ | 29 | — | 5 | — | 48 | 17 | 11 | 81 |
| Totals | ♀ | 150 | — | 36 | 16 | 176 | 88 | 46 | 277 |
| | ♂ | 116 | — | 31 | — | 227 | 86 | 48 | 341 |
| Grand totals | | 266 | — | 67 | 16 | 403 | 174 | 94 | 618 |

(*B*) *Vortex female by heterozygous male.*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M417 | ♀ | 34 | — | — | 4 | 44 | — | — | 62 |
| | ♂ | 25 | — | — | — | 33 | — | — | 45 |
| M418 | ♀ | 14 | — | — | 9 | 18 | — | — | 31 |
| | ♂ | 13 | — | — | — | 18 | — | — | 35 |
| Grand totals | | 86 | — | — | 13 | 113 | — | — | 173 |

(*C*) *Dichaete vortex hairless female ex M389 by vortex male.*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| M450 | ♀ | 54 | 2 | 18 | 23 | — | 22 | — | 36 |
| | ♂ | 56 | — | 27 | — | 9 | 23 | — | 60 |
| Grand totals | | 110 | 2 | 45 | 23 | 9 | 45 | — | 96 |

## THE ISOLATION OF AN ADDITIONAL VORTEX INTENSIFIER

During the course of all these later experiments a sharp outlook was kept for the occurrence of dichaete vortex males such as had been found in the first experiments with the mutant (table 1). In only one of the many cultures was such a male recorded (M446, table 7). This culture was likewise exceptional in the high number of dichaete vortex females

of the heterozygous type, over 40 percent of the dichaete females being vortex instead of under 20 percent.

Here was an opportunity to determine whether this male was of a different genetic constitution from ordinary dichaete males which do not show vortex. The male was out-crossed to a vortex female from stock. The absence of dichaete vortex males in the sons of the dichaete vortex male (M508, table 14) proves that he had not been a crossover; that is, that he was not homozygous for vortex III, for in that case practically all of his offspring should have been vortex.

The $F_1$ culture was exceptional in that there was a very high proportion of vortex among the dichaete females, just as had been the case in the parent culture 446. On the other hand none of the dichaete males showed vortex. So that it may be concluded provisionally that a modifying gene was present which was partially dominant among the females and not obviously dominant among the males. This difference is another expression of the already noted sex-limitation of the vortex character.

TABLE 14

*Selection for dichaete vortex males.*

| No. | Parentage | | $D$ | $\frac{D}{v_o}$ | $v_o$ | $\frac{\cdot}{+}$ |
|---|---|---|---|---|---|---|
| M508 | $v_o$♀ × $Dv_o$♂ ex 446 | ♀ | 16 | 25 | 48 | — |
| | | ♂ | 36 | — | 16 | 3 |
| M550 | $2Dv_o$♀ × $2D$♂ ex 508 | ♀ | 16 | 22 | 14 | — |
| | | ♂ | 33 | 8 | 15 | — |
| M608 | $3Dv_o$♀ × $3Dv_o$♂ 550 | ♀ | 37 | 82 | 30 | — |
| | | ♂ | 68 | 11 | 34 | — |

Culture 508 was so similar to 446 that it seemed probable that they were of the same constitution and that the single dichaete vortex male of 446 was a case of the dominance of the modifier, here effective even in the male. Another indication of this dominance is the suppression to a large extent of the changing over of vortex into wild-type in 446.

An $F_2$ culture was raised from two of the dichaete vortex females mated to two of the dichaete males from 508. While among the dichaete females the proportion of vortex was no higher than in $F_1$, among the males 8 dichaete vortex males occurred in a total of only 41 dichaetes. These males are presumably to be looked upon as homozygous for the modifier. Three such males were crossed to dichaete vortex sisters and

the succeeding generation (M608) was characterized by the highest proportion of dichaete vortex yet observed. More than two-thirds of the dichaete females were vortex, indicating that more flies were homozygous for the modifier than in the previous cross. Another feature of these last two cultures was the absence of wild-type males in contrast to their usual occurrence in cultures free from the modifier. Thus the grade of vortex in all of its types has been raised to a high level by the action of the modifier, but this level is consistently higher in the female than in the male.

The gene for the modifier is known not to be in the third chromosome, unless very removed from dichaete, and the probabilities are that it is in the second chromosome.

The new experiments removed the suspicion that the inheritance of vortex depended upon some unusual chromosome condition. Thus, sterility did not appear in the new experiments, and its occurrence in the first experiments must have been a separate phenomenon. The locus of vortex III proved to be close to that of dichaete and not at the middle of the chromosome as required for "transposition". The other doubtful points have likewise fallen in line with a plural gene explanation.

### THE MUTANT CHARACTER, FLIPPER

In culture 367 (table 5) a very small wild-type female was found and tests were made to determine whether she was genetically a dwarf or simply was exceptionally small because of some accident of development. In $F_2$ from a cross to vortex male no dwarfs reappeared, but a new mutant character appeared in the culture (M466). This new mutation resembled the sex-linked mutation club (see MORGAN and BRIDGES, Carnegie Publication No. 237 for figure). The whole fly was under-sized and was of shrunken appearance. The surface retained a wet appearance. The most obvious feature was the wing which remained in the folded condition in which they were when the fly emerged from the pupa case, and did not expand as wings normally do. These compact wings were held out and curved downwards like flippers.

When these flies began to appear it was observed that most of them were at the same time vortex. Counts were made which show that there was strong linkage betwen vortex and flipper (M466, table 15).

Several attempts were made to mate these flies together in order to obtain a stock of the mutation. All these matings failed except one, which gave 4 flipper females, 1 flipper male, and 1 wild-type male. Probably the wild-type male resulted from non-virginity of the mother.

TABLE 15

*$F_2$ results from the cross of vortex flipper to wild.*

| | Wild-type | Vortex | Vortex flipper | Flipper |
|---|---|---|---|---|
| M466 | 190 | 17 | 32 | 3 |
| M723 | 232 | 14 | 40 | 7 |
| M724 | 229 | 19 | 25 | 5 |
| Total | 651 | 50 | 97 | 15 |

Because of the failure of all these matings the character flipper was lost, but it reappeared later in another culture in which vortex was used. In this case also quite extensive matings were made between vortex flipper females and their wild-type brothers. In one case only the mating produced a few wild-type offspring, from which two $F_2$ cultures were raised (723 and 724, table 15).

A calculation of the position of flipper was made on the basis of the three $F_2$ cultures of table 15. The flipper class (15) is a crossover class corresponding to the non-crossover vortex flipper class (97). Likewise the vortex class is a crossover class which corresponds to the compound wild-type class. The wild-type class is constituted from 3 non-crossover and 2 crossover classes ($3n + 2x$). The non-crossover class corresponding to the crossover class is calculated as 184 individuals. The total results give 65 crossovers to a total of 346 individuals, or 18.7 percent of crossing over. The amount of crossing over between vortex and flipper is so large that it is improbable that the locus of flipper is to left of vortex since vortex is itself only 10 from the left end of the known chromosome. Flipper can be located approximately at a position 18.7 to the right of vortex or at 28.3 to the right of star.

## SUMMARY

The foregoing experiments have shown that the character vortex is dependent upon or is modified by four mutant genes.

Of these genes the most essential one, without which the character is never known to have appeared, is situated in the second chromosome at a position 9.6 units to the right of star. However, this second-chromosome gene is by itself insufficient for the production of the vortex character.

The gene second in effectiveness is situated in the third chromosome very close to the locus of dichaete (11.7). This gene likewise is unable

to cause any development of the vortex character when acting alone. But in flies homozygous for vortex II, heterozygosity for vortex III enables about 20 percent of the heterozygous females to show the vortex character although no male of this constitution can show the character. Flies homozygous for both vortex III and vortex II are, if females, practically invariably vortex, while if males they are vortex except that toward the end of old cultures a small proportion of genetically vortex males of this homozygous type "change over" into wild-type. The usual stock of vortex is of this bigenic constitution.

During the experiments a dichaete stock was isolated in which a third gene was present which contributed to the development of the vortex character. In this stock a majority of the females homozygous for vortex II and heterozygous for vortex III showed the character, instead of only about 20 percent as in stocks in which this modifier is not present. A slightly greater percent of such females showed vortex when homozygous for the new modifier. In heterozygous condition this new modifier was almost without effect upon males of the heterozygous type, but in homozygous condition it made vortex show in a considerable proportion of the flies homozygous for vortex II and heterozygous for vortex III, while it eliminated the changing over of homozygous vortex II vortex III flies into wild-type flies. The locus of this modifier is probably in the second chromosome.

In the experiments involving streak a special relation between streak and the vortex was discovered. The vortex character was prevented from developing in streak flies even though such flies were homozygous for both vortex II and vortex III.

However, in the same experiments a special modifier was detected which to a considerable extent reversed this inhibition by streak. This modifier was a dominant situated in the second chromosome quite close to the locus of streak and probably to the right. The streak flies in which the vortex character appeared through the action of the modifier showed a type of vortex different from the usual one.

Throughout all of these experiments and in the various types of vortex a very striking fact was apparent, namely, that the grade of the vortex character and the proportion of flies showing that character was higher in the females than in males of the same genetic constitution.